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Cover image: A. Bareogonalosjezoensis female ovipositing on a leaf of Atremisia montana (Courtesy:Prof.Seike Yamane)

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First Record of Genus Pristomyrmex Mayr 1866 (Hymenoptera: Formicidae) from India

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Abstract

The genus Pristomyrmex Mayr, 1866 is recorded for the first time from India. The species Pristomyrmex sulcatus Emery, 1895 has been collected from North-East Himalaya.

Keywords: Myrmicinae, Pristomyrmex sulcatus, India, North-East Himalaya.

Introduction

The ant genus Pristomyrmex Mayr comprises of 58 extant valid species restricted to the Old World tropics (Sarnat and Economo, 2013, Bolton, 2014). Most of the species occur primarily in the Oriental region, but few endemic species have been reported from Australia, Africa and Fiji. In addition, the genus has also been reported from Mauritius and temperate regions of China, Korea and Japan (Wang, 2003, Guenard et al. 2010 and Bolton, 2014). A comprehensive taxonomic revision of the genus was carried by Wang (2003). Other taxonomic contributions to the genus include, a review of the Philippine Pristomyrmex with three new descriptions and a key to the species (Zettel, 2006), a key to the Taiwan species (Terayama, 2009) and more recently report of a new species from Fiji (Sarnat and Economo, 2013).

As mentioned by Wang (2003) most of the Pristomyrmex species forage as predators and scavengers. The nests can be found in soil, litter rotten logs, in plants roots etc.

From India, the genus Pristomyrmex is reported for the first time. The species Pristomyrmex sulcatus Emery, 1895 is collected from North-East Himalaya. Earlier, this species have been recorded from China, Nepal, Thailand, Malaysia and Myanmar.

Material Examined

3 workers, India, East Sikkim, Rorathang, 587m, 27°11'49.91"N, 88°36'12.44"E, 12.vi. 2012.

Figs. 1-3: Pristomyrmex sulcatus Emery, 1895 1. Head, full face view; 2. Body, lateral view; 3. Body, dorsal view.

(Online: 23-February-2014; Print: 9-January-2015)

Fig. 4: Map depicting collection site (marked by asterisk)

Ecology

The specimens were collected by Winkler's extractor from an undisturbed dense secondary forest. The thickness of leaf litter was about 4 inches. The floor of the forest receives limited sun light. The maximum temperature of the area is 28°C, minimum -1°C and rainfall 325cm per annum.

Acknowledgments

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References

Bolton, B. 2014. An online catalogue of the ants of the world. http://antcat.org.(accessed 21 February 2014)

Guénard, B., Weiser, M. D. and Dunn, R. R. 2010. Global generic richness and distribution: new maps of the world of ants with examples of their use in the context of Asia. Asian Myrmecology 3:21-28.

Sarnat, E. M. and Economo, E.P. 2013.Pristomyrmex tsujii sp. n. and P. mandibularisMann (Hymenoptera, Formicidae) from Fiji. ZooKeys 340: 43–61.

Terayama, M. 2009. A synopsis of the family Formicidae of Taiwan (Insecta: Hymenoptera). Research Bulletin of Kanto Gakuen University Liberal Arts 17: 81–266.

Wang, M. 2003. A monographic Revision of ant genus Pristomyrmex (Hymenoptera: Formicidae). Bulletin of the Museum of Comparative Zoology 157(6): 383-542.

Zettel, H. 2006. On the ants (Hymenoptera: Formicidae) of the Philippine Islands: I. The genus Pristomyrmex Mayr, 1866. Myrmecologische Nachrichten 8: 59–68.

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Proceratium melinum (Roger, 1860) (Hymenoptera: Formicidae) in Romania: a new record of the species after a century

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Abstract

Proceratium melinum (Roger, 1860) is a hypogaeic ant species that occurs in southern Europe. Very little is known about the ecology and the distribution of this ant. In Romania there have been only two previous records from almost a century ago. We present a new record of this species and some insights regarding its ecology.

Keywords: hypogaeic ants, distribution, Europe, habitat requirements.

Introduction

Proceratium ants are mostly hypogaeic (subterranean), specialized predators on spider eggs and other arthropods (Brown, 1958 a, b, 1974, 1980; Dietrich, 2004; Fisher, 2005). Proceratium melinum (Roger, 1860) is one of the three species of this genus that occurs in Europe. The known distribution of P. melinum includes: Albania, Austria, Bulgaria, Croatia, Czech Republic, France, Greece, Hungary, Italy, Israel, Malta, Macedonia, Montenegro, Romania, Russia, Slovenia, Spain, Turkey and Ukraine (Fig. 1, see supplementary material for references). With the exception of this distributional information and some aspects regarding its biology, the species is unstudied.

The checklist of the Romanian ant fauna contains 109 species (Markó et al. 2006; Czechowski et al. 2012; Czekes et al. 2012). However, considering the geographical location of Romania and the greater number of known ant species from neighboring countries, Romania's ant fauna is likely under sampled (Czechowski et al. 2012). Moreover, data is particularly scarce for cryptic, sub-Mediterranean and parasitic ant species (Markó et al. 2006; Markó, 2008). Currently six ant species with cryptic lifestyles are known to occur in Romania: Cryptopone ochracea (Mayr, 1855), Hypoponera punctatissima (Roger, 1859), Ponera coarctata (Latreille, 1802), P. testacea Emery, 1895, Pyramica baudueri (Emery, 1875) and Proceratium melinum. Except Ponera coarctata (see Csősz, 2003, Markó et al., 2006), these species have been collected from only a few sites. Pyramica baudueri is known from Arad County (Markó, 2008), whereas Hypoponera punctatissima was sampled in the surroundings of Bucharest (Paraschivescu, 1974). Cryptopone ochracea is known from Bucharest (Montandon and Santschi, 1910) and Băile Herculane (Csősz, 2003). Finally, Ponera testacea is known from five sites: Bucharest (Montandon and Santschi, 1910), Deva, Sibiu and Cluj-Napoca (Csősz and Seifert, 2003) and Arad County (Markó, 2008).

Despite being reported from southern Europe, the distribution and biology of P. melinum are poorly known. In Romania, probably due to its cryptic lifestyle and the lack of appropriate collection methods, the species

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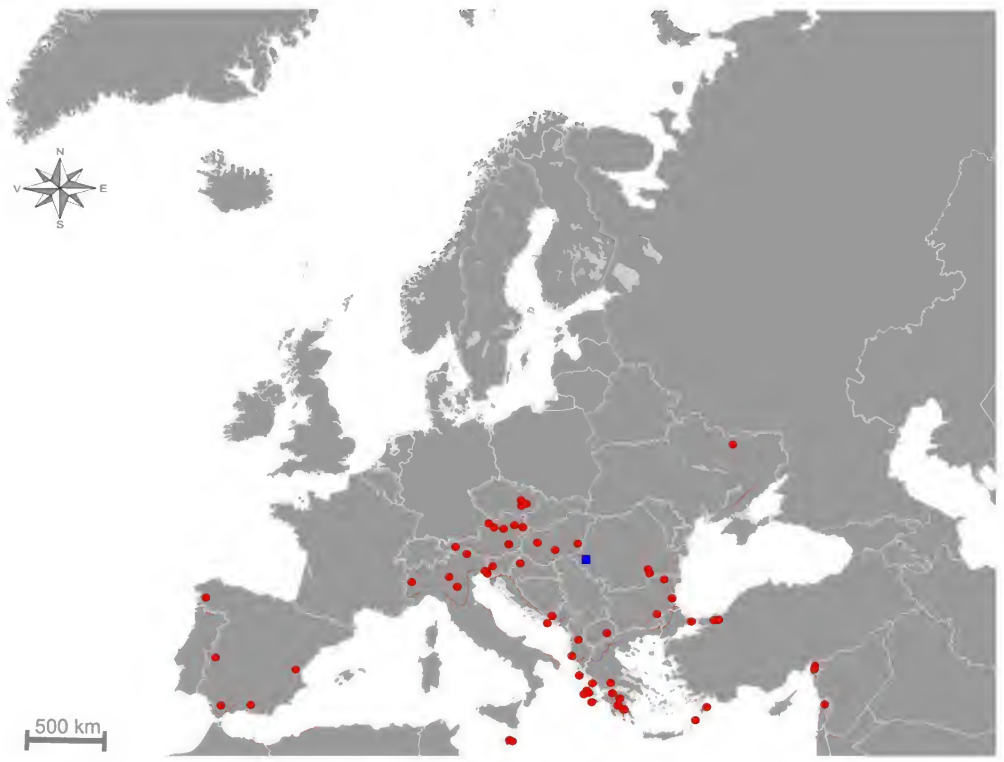

Fig.1: The known distribution of Proceratium melinum in Europe (red circles - published literature, blue square – new record).

was previously recorded only from two localities: Comana Vlasca (one worker– Montandon and Santschi, 1910) and Bucharest (two queens, collected in 1897 – Lomnicki, 1922). Herein we report a new record for P. melinum.

Materials and Methods

One worker (Figs. 2-5) and one queen (Fig. 6) of Proceratium melinum (Roger, 1860) were collected by pitfall traps during a myrmecological survey in Mlaştinile Satchinez Nature Reserve (Fig. 1, N 45.937706, E 21.036433, ca. 90 m a.s.l., Timiş County, Western Romania) on 12.09.2012. The species is easy to distinguish from other ant species in Romania, due to obvious morphological characters of the genus: a large and vaulted abdomen, with the tergite of the second abdominal segment strongly arched so that the rest of the segments are pointed anteriorly, a dentate mandible and the apically incrassate funiculus (Onoyama and Yoshimura, 2002; Csősz, 2003). The specimens are deposited in the Natural History Museum of the Brukenthal National Museum, Sibiu, Romania. In addition, a distribution map was compiled based on the available data (precise published records of the species – see supplementary material). However, in some cases, collecting sites were identified only as larger areas (e.g. mountain or steppe). Thus, the map of the species does not feature these locations.

Ecology

According to Brown (1958b): "Proceratium ants nest consists of small rounded chambers hollowed out of soft rotten wood or in the soil; toward the cooler limits of the range, particularly in North America, nests

Fig. 2: Proceratium melinum (Roger, 1860) - lateral view of worker (photo: S. Török)

Fig. 3-5: Proceratium melinum (Roger, 1860) worker from AntWeb (CASENT0907204), 3. head in full face view; 4. lateral view; 5. dorsal view

.

Fig. 6: Proceratium melinum (Roger, 1860) - lateral view of queen (photo: S. Török).

and foraging workers are found under deepest site is usually in forest shade, in old moist gardens, or similar habitats that are constantly moist".

We collected the ants from an agricultural field, which is located near the swamp habitats of the reserve. In the spring the fields are usually flooded. Our collection in this habitat is consistent with Brown (1958b). Moreover, most of the published records are in agreement with the species humidity requirements due to frequently sampling from wet habitats (e.g. river valleys).

Additionally, Masuko (1986), Baroni Urbani and de Andrade (2003) observed a form of vampirism (e.g. the queens drinking the haemolymph of the larvae) in the species P. japonicum, P. itoi, and P. watasei and other hypogaeic ants. Due to the close phylogenetic rocks instead of in rotten wood. The nest relationship with the latter species, P. melinum may exhibit similar behavior (Baroni Urbani and de Andrade, 2003). This bizarre form of non-destructive cannibalism could be regarded as an adaptation related to the lack of social food transfer (Masuko, 1986).

In conclusion, we believe the use of varied collecting methods including methods specific for cryptic species (Wilkie et al. 2007; Schmidt and Solar, 2010) would improve both occurrence and distribution information for ants in Romania. Additionally, investigating the habitat specificity of P. melinum would define this ant's local distribution. And finally, this collection raises some interesting questions concerning the ecology of P. melinum which may increase our understanding of the relationships within this genus.

Table-1: Supplementary material: List of the published records of Proceratium melinum (*– not shown in map).**

Country	Sites	Source
Albania	Vlora	Baroni Urbani and de Andrade, 2003
Albania	Tirana	Baroni Urbani and de Andrade, 2003
Austria	Neustift	Dietrich, 2004
Austria	Illmitz	Dietrich, 2004
Austria	Sankt Pölten	Dietrich, 2004
Austria	Graz	Dietrich, 2004
Austria	Freinberg	Dietrich, 2004
Austria	Steyregg	Dietrich, 2004
Austria	Purgstall	Dietrich, 2004
Bulgaria	Dobrudzha	Lapeva-Gjonova et al., 2010
Bulgaria	Svilengrad	Lapeva-Gjonova et al., 2010
Bulgaria	Burgas	Lapeva-Gjonova et al., 2010
Croatia	Krapina	Csősz, 2003
Croatia	Konavle	Bračko, 2006
Czech Republic	Moravia	Werner and Wiezik, 2007
Czech Republic	Brno	Werner and Wiezik, 2007
Czech Republic	Kremsier	Baroni Urbani and de Andrade, 2003
France	***Pirines	Casevitz-Weulersse and Galkowski, 2009
Greece	Dodecanese	Borowiec and Salata, 2012
Greece	Cephalonia, Sami	Baroni Urbani and de Andrade, 2003
Greece	Valsamata	Baroni Urbani and de Andrade, 2003
Greece	Argostoli	Baroni Urbani and de Andrade, 2003
Greece	Lakonia, Sparta	Baroni Urbani and de Andrade, 2003
Greece	Ithaki, Anoghi	Baroni Urbani and de Andrade, 2003
Greece	Zante	Baroni Urbani and de Andrade, 2003
Greece	Vasilikon	Baroni Urbani and de Andrade, 2003
Greece	Achaia, Kastritsion	Baroni Urbani and de Andrade, 2003
Greece	Messe, Analipois	Baroni Urbani and de Andrade, 2003
Greece	Ionians	Borowiec and Salata, 2012
Greece	Rhodes, Profitis Ilias	Baroni Urbani and de Andrade, 2003
Greece	Peloponnese	Borowiec and Salata, 2012
Greece	Sterea Ella	Borowiec and Salata, 2012
Greece	Corfu	Baroni Urbani and de Andrade, 2003
Hungary	Kiskunhalas	Csősz, 2003
Hungary	Révfülöp	Csősz, 2003
Hungary	Gyula	Csősz, 2003
Israel	Golan Heights	Vonshak and Ionescu, 2009
Italy	Bressanone	Baroni Urbani and de Andrade, 2003

Italy	Emilia, Castelvetro	Baroni Urbani and de Andrade, 2003
Italy	Triest	Baroni Urbani and de Andrade, 2003
Italy	Coazze	Baroni Urbani and de Andrade, 2003
Italy	Hermada-Duino	Baroni Urbani and de Andrade, 2003
Italy	Bardolino, Verona	Baroni Urbani and de Andrade, 2003
Macedonia	Kos Hill, Demir Kapija	Karaman, 2009
Malta	Birkirkara	Schembri and Collingwood, 1981
Malta	Chadwick	Schembri and Collingwood, 1981
Montenegro	Castelnuovo –Herceg-Novi	Baroni Urbani and de Andrade, 2003
Romania	Comana Vlască	Montandon and Santschi, 1910
Romania	Bucharest	Lomnicki, 1922
Romania	**Satchinez**	**New record**
Russia	***don steppe	Baroni Urbani and de Andrade, 2003
Slovenia	Skrilje	Bračko, 2007
Spain	Caceres	García et al., 2009
Spain	Castellon	Baroni Urbani and de Andrade, 2003
Spain	Granada	Baroni Urbani and de Andrade, 2003
Spain	Pontevedra	Baroni Urbani and de Andrade, 2003
Spain	Sevilla	García et al., 2009
Turkey	Adapazan-Pamukova	Kiran and Karaman, 2012
Turkey	Antarkya, Yoselkent	Kiran and Karaman, 2012
Turkey	Sakarya	Kiran and Karaman, 2012
Turkey	Bebek, Istambul	Kiran and Karaman, 2012
Turkey	Hatay	Baroni Urbani and de Andrade, 2003
Ukraine	Korobotschkino, Kharkiv	Baroni Urbani and de Andrade, 2003

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References

Baroni Urbani, C. and de Andrade, M.L. 2003. The ant genus Proceratium in the extant and fossil record (Hymenoptera: Formicidae). Museo Regionale di Scienze Naturali Monografie (Turin) 36:1-492.

Borowiec, L. and Salata, S. 2012. Ants of Greece–checklist, comments and new faunistic data (Hymenoptera: Formicidae). Genus 23(4): 461-563.

Bračko, G. 2006. Review of the ant fauna (Hymenoptera: Formicidae) of Croatia. Acta Entomologica Slovenica 14(2): 131-156.

Bračko, G. 2007. Checklist of the ants of Slovenia (Hymenoptera: Formicidae). Natura Sloveniae 9(1): 15-24.

Brown, W.L. Jr. 1958a. Predation of arthropod eggs by the ant genera Proceratium and Discothyrea. Psyche 64: 115.

Brown, W.L. Jr. 1958b. Contributions toward a reclassification of the Formicidae. II. Tribe Ectatommini (Hymenoptera). Bulletin of the Museum of Comparative Zoology 118: 173-362.

Brown, W.L. Jr. 1974. A remarkable new island isolate in the genus Proceratium (Hymenoptera: Formicidae). Psyche 81: 70-83.

Brown, W.L. Jr. 1980. A remarkable new species of Proceratium, with dietary and other notes on the genus (Hymenoptera: Formicidae). Psyche 86: 337-346.

Casevitz-Weulersse, J. and Galkowski, C. 2009. Liste actualisée des Fourmis de France (Hymenoptera, Formicidae). Bulletin de la Société Entomologique de France 114(4): 475-510.

Csősz, S. 2003. A key to the Ponerinae species of the Carpathian Basin (Hymenoptera: Formicidae). Annales Historico-Naturales Musei Nationalis Hungarici 95: 147-160.

Csősz, S. and Seifert, B. 2003. Ponera testacea Emery, 1895 stat n. – a sister species of P. coarctata (Latreille, 1802) (Hymenoptera, Formicidae). Acta Zoologica Academiae Scientiarum Hungaricae 49(3): 201-214.

Czechowski, W., Radchenko, A., Czechowska, W. and Vepsäläinen, K. 2012. The ants of Poland with reference to the myrmecofauna of Europe. Fauna Poloniae 4. Warsaw: Natura Optima Dux Foundation, 496 pp.

Czekes, Z., Radchenko, A.G., Csősz, S., Szász-Len, A., Tăuşan, I., Benedek, K. and Markó, B. 2012. The genus Myrmica Latreille, 1804 (Hymenoptera: Formicidae) in Romania: distribution of species and key for their identification. Entomologia Romanica 17: 29-50.

Dietrich, C.O. 2004. Die Krummameise, Proceratium melinum (Roger 1860), ein unauffälliger und bemerkenswerter Einwanderer in Österreich (Hymenoptera: Formicidae).Wissenschaftliche Mitteilungen aus dem Niederösterreichischen Landesmuseum 16: 7-32.

Fisher, B.L. 2005. A new species of Discothyrea Roger from Mauritius and a new species of Proceratium Roger from Madagascar (Hymenoptera: Formicidae). Proceedings of the California Academy of Sciences 56: 657-667.

García, F., Espadaler, X. and Gómez, K. 2009. Primera cita de Amblyopone impressifrons (Emery, 1869) para la Península Ibérica y de Proceratium melinum (Roger, 1860) para Cataluña (Hymenoptera, Formicidae). Boletín de la SEA 45: 357-360.

Karaman, M.G. 2009. An introduction to the ant fauna of Macedonia (Balkan Peninsula), a check list (Hymenoptera, Formicidae). Natura Montenegrina 8(3): 151-162.

Kiran, K. and Karaman, C. 2012. First annotated checklist of the ant fauna of Turkey (Hymenoptera: Formicidae). Zootaxa 3548: 1-38.

Lapeva-Gjonova, A., Antonova, V., Radchenko, A.G. and Atanasova, M. 2010. Catalogue of the ants (Hymenoptera, Formicidae) of Bulgaria. ZooKeys 62: 1-124.

Lomnicki, J. 1922. Przyczynek do opisu królowej mrówki powolnicy europejskiej (Sysphincta europaea For.). Polskie pismo entomologiczne 1: 3-4.

Markó, B. 2008. Pyramica baudueri (Emery, 1875) – a new ant species (Hymenoptera: Formicidae) for the Romanian fauna. Fragmenta Faunistica 51(2): 101-106.

Markó, B., Sipos, B., Csősz, S., Kiss, K., Boros, I. and Gallé, L. 2006. A comprehensive list of the ants of Romania (Hymenoptera: Formicidae). Myrmecologische Nachrichten 9: 65-76.

Masuko, K. 1986. Larval hemolymph feeding: a non-destructive parental cannibalism in the primitive ant Amblyopone silvestrii Wheeler (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 19: 249-255.

Montandon, A.L. and Santschi, F. 1910. Contributions á la faune entomologique de la Roumanie. Formicides. Bulletin de la Société Roumaine des Sciences 19: 648–654. Bulletin de la Société Roumaine des Sciences 19: 648–654.

Onoyama, K. and Yoshimura, M. 2002. The ants of the genus Proceratium (Hymenoptera: Formicidae) in Japan. Entomological Science 5(1): 29-50.

Paraschivescu, D. 1974. Die Fauna der Formiciden in dem Gebiet um Bukarest. Travaux du Muséum d'Histoire Naturelle "Grigore Antipa" 15: 297-302.

Schmidt, F.A. and Solar, R.R.C. 2010. Hypogaeic pitfall traps: methodological advances and remarks to improve the sampling of a hidden ant fauna. Insectes Sociaux 57(3): 261-266.

Schembri, S.P. and Collingwood, C.A. 1981. A revision of the myrmecofauna of the Maltese Islands (Hymenoptera, Formicidae). Annali del Museo Civico di Storia Naturale Giacomo Doria (Genova) 83: 417-442.

Vonshak, M. and Ionescu-Hirsch, A. 2009. A checklist of the ants of Israel (Hymenoptera: Formicidae). Israel Journal of Entomology 39: 33-55.

Werner, P. and Wiezik, M. 2007. Vespoidea:Formicidae (mravencovití). Acta Entomologica Musei Nationalis Pragae 11: 133-164

Wilkie, K.T.R., Mertl, A.R. and Tranielo, J.F.A. 2007. Biodiversity below ground: probing the subterranean ant fauna of Amazonia. Naturwissenschaften 94: 392-395.

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Biology of the harpactorine assassin bug, Panthous bimaculatus Distant (Hemiptera: Reduviidae) on three different diets

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Abstract

The biology of the predatory reduviid, *Panthous bimaculatus* Distant, when maintained at a temperature of 32 (±2) °C, 75±5 % relative humidity and 12 (±1) hour's photoperiod on three different diets, namely, *Corcyra cephalonica*, artificial diet and *Spodoptera litura* was investigated. The incubation period was 21.3 ± 0.86, 21.00 ± 0.00, 23.00 ± 0.00 when fed on *Corcyra cephalonica*, artificial diet and *Spodoptera litura* respectively. The duration of development for the first, second, third, fourth and fifth instars fed with *C. cephalonica* was 13.47 ± 0.44, 11.56 ± 0.34, 16.24 ± 0.77, 15.24 ± 0.96 and 18.57 ± 2.53. With artificial diet, the readings were 15.76 ± 1.36, 17.00 ± 3.27, 24.25 ± 5.08, 15.00 ± 0.00 and 17.20 ± 1.57. The developmental time with regard to the feed *S. litura* was 13.67 ± 0.55, 11.97 ± 0.60, 15.86 ± 1.04, 15.36 ± 1.47 and 16.25 ± 1.82.

Keywords: *artificial diet, biology, Corcyra cephalonica, Panthous bimaculatus, Spodoptera litura.*

Introduction

The hunter reduviid, Panthous bimaculatus Distant is a potential predator and important biological control agent of many economically important pests. Reduviids in general are predacious insects that have proven to be of agricultural importance (Ambrose, 1999). They constitute one of the largest groups of predacious insects with approximately 6,800 described species (Hwang and Weirauch, 2012). The phenomenal success of their group can be solely attributed to their readily adaptive nature to hostile environmental conditions in combination with a wide range of prey capture strategies. The extreme environments they have seen to thrive in include deserts and rainforests (Ryckman, 1954; Miller, 1959). Many of these bugs are found to occur in large numbers on crevices in tree barks, shrubs and on foliage in agro ecosystems (Readio, 1927; Miller, 1953; Louis, 1974; Ambrose, 1999; Subramaniyan and Kitherian, 2012). As stated by Ambrose et al. (1999), these voracious predators due to their polyphagous nature have the potential to serve as valuable biocontrol agents in situations where a large number of various insects are present rather than in areas which are heavily infested by a single pest species.

Their unprecedented success over the last few hundred years in every microhabitat or niche possible is primarily due to the morphological, physiological adaptations with respect to feeding in combination with their extra – oral digestion facilitated by their venomous saliva (Edwards, 1961). Hence, studies on reduviidae can throw light on pest control by natural methods and can yield a new perspective to agricultural scientists in the evolution of eco-friendly pest management technologies. For this purpose, they need to be conserved and augmented (Schaefer and Ahmad, 1987). Conservation and augmentation can be achieved only with a comprehensive and elaborate understanding of their biology. In spite of their significant role in the construction and implementation of natural methods in pest control, there is ample literature on the habitat and ecological specialization of only some species of reduviids; most other species have been untouched. Hence, this study attempts to study the biology of one such untouched

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assassin bug, Panthous bimaculatus on three different diets.

Materials and methods

Laboratory colonies of the predator P. bimaculatus were established from adult male and female insects that were collected from Marunthuvazh Malai scrub jungle (latitude 8°8'11"N 77°30'47"E) in Kanyakumari District, Tamil Nadu, South India. They were separated into three groups and reared. Each group was maintained on a different food source namely, larvae of rice meal moth Corcyra cephalonica, artificial diet and Spodoptera litura. These predatory insects were kept at a room temperature of 32 (±2) °C, 75±5 % relative humidity and 12 (±1) hour's photoperiod. The adults were placed in separate containers and were allowed to mate. The containers were carefully examined at regular intervals to record the number of eggs laid. The eggs thus laid were shifted to petri dishes (9.2 x 2.0 cm) with wet cotton swabs. The moist cotton serves to maintain optimum humidity. The cotton swabs were changed at regular time intervals. The predators were reared in the laboratory for two generations to find out the incubation period, stadial period, nymphal mortality, longevity and sex ratio.

Types of Diets

Corcyra cephalonica also called rice meal moth larvae was reared in the laboratory. 2 kg of wheat flour, 250 grams of coarsely powdered groundnut, a pinch of streptomycin, 10 grams of yeast crystals, and 2 cc of Corcyra cephalonica eggs obtained from Sun Agro Biosystem Pvt Ltd, Porur were mixed well and the mixture was left undisturbed for 60 days after which the grown larva were collected to be used as feed for Panthous bimaculatus.

For the preparation of artificial diet, 200 gram beef liver extract, 200 gram fatty ground beef, 24 ml sucrose solution (5%), 1 gram ascorbic acid, 2 gram Wesson's salt mixture, 20 gram fresh egg yolk. All the ingredients were first blended with a kitchen blender until uniformly mixed. The liquid mixture was used fresh or deep frozen in small containers (De Clercq and Degheele, 1992). Cotton balls dipped in the liquid mixture were offered to the predator.

Spodoptera litura, are serious agricultural pest were collected from groundnut plantations in Kanyakumari District to be used as feed for the reduviid predator.

Biology

The Panthous bimaculatus laid pale yellow eggs singly, scattered and at times in batches, just like those of Sphedanolestes variabilis (Ambrose et al., 2009) approximately after 21.3 ± 0.86 days, 21.00 days and 23.00 days when fed on Corcyra cephalonica, artificial diet and Spodoptera litura respectively. The incubation period was much greater than that of Rhynocoris marginatus (6.81±0.10 days) (Sahayaraj and Paulraj, 2001) and Sinea complexa (15.5 days) (Swadener and Yonke, 1973) but same as that of Sycanus reclinatus (22 days) (Vennison and Ambrose, 1992). The eggs were pale yellow at the time of laying. After a couple of hours the fertilized eggs acquired a darker shade of brownish yellow. After 5 to 6 days, the fertilized eggs became brownish yellow. After almost 13 days, they turned slightly reddish with the presence of a red chorion and a few days before hatching they donned a bright red color. The hatch percentage was 100. The newly hatched nymphs were delicate and fragile and their color darkened 8 to 10 hours after hatching. It was also noticed that the new hatchlings preferred small and sluggish prey.

The newly emerged hatch outs which were pale amber in colour acquired a darker shade within a few hours of hatching. This colour change in nymphs just after hatching is also observed in Sycanus reclinatus, where the nymphs change from pale-ochraceous to dark-ochraceous a few hours after hatching (Vennison and Ambrose, 1992).

Ambrose (1999), has recorded the preoviposition period of Rhynocoris marginatus at 33.30 days, R. kumarii at 26 days, R. longifrons at 11.80 days, Ectrichodinae at 7.0 days, Salyavatinae at 6.7 days, Stenopodainae at 14.3 days, Triatominae at 14.83 days, Reduviinae at 30.4 ± 14.71 days and Peiratinae at 16.86 ± 4.36 days. The preoviposition period of Panthous bimaculatus at 12.3 days falls closer to Rhynocoris longifrons, followed by

Results:

Table 1: Incubation period, nymphal developmental time (days), sex ratio of P. bimaculatus reared on Corcyra cephalonica

Name of the predator		Panthous bimaculatus
Reared on		Corcyra cephalonica
Incubation period		21.3±0.86
Stadial period	I	13.47 ± 0.44
	II	11.56 ± 0.34
	III	16.24 ± 0.77
	IV	15.24 ± 0.96
	V	18.57 ± 2.53
Adult		69.00 ± 9.45
Sex Ratio		1:0.71

Table 2: Survival rate in % of P. bimaculatus, reared on Corcyra cephalonica

Name of the predator		Panthous bimaculatus
Reared on		Corcyra cephalonica
Hatchability		100
Nymphal survival	I	85.71
	II	78.57
	III	60.71
	IV	44.64
	V	12.5
Total		12.00

Table 3: Incubation period, nymphal developmental time (days), sex ratio of P. bimaculatus reared on artificial diet

Name of the predator		Panthous bimaculatus
Reared on		Artificial diet
Incubation period		21.00 ± 0.00
Stadial period	I	15.76 ± 1.36
	II	17.00 ± 3.27
	III	24.25 ± 5.08
	IV	15.00 ± 0.00
	V	17.20 ± 1.57
Adult		67.00 ± 2.43
Sex Ratio		1:0.65

Table 4: Survival rate in % of P. bimaculatus, reared on artificial diet

Name of the predator		Panthous bimaculatus
Reared on		Artificial diet
Hatchability		100
Nymphal survival	**I**	70.00
	II	53.33
	III	42.36
	IV	23.33
	V	16.36
Total		16.03

Table 5: Incubation period, nymphal developmental time (days), sex ratio of P. bimaculatus reared on Spodoptera litura

Name of the predator		Panthous bimaculatus
Reared on		Spodoptera litura
Incubation period		23.00 ± 0.00
Stadial period	**I**	13.67 ± 0.55
	II	11.97 ± 0.60
	III	15.86 ± 1.04
	IV	15.36 ± 1.47
	V	16.25 ± 1.82
Adult		68.88 ± 2.97
Sex Ratio		1:0.55

Table 6: Survival rate in % of P. bimaculatus, reared on Spodoptera litura

Name of the predator		*Panthous bimaculatus*
Reared on		*Spodoptera litura*
Hatchability		100
Nymphal survival	**I**	78.00
	II	60.00
	III	44.00
	IV	22.00
	V	15.30
Total		15.00

Stenopodainae and Triatominae.

The sex ratio observed in lab bred P. bimaculatus on the three different diets were 1:0.71, 1:0.65 and 1:0.55 respectively. However, it is interesting to note that an unbiased sex ratio was seen in laboratory reared reduviid bugs such as, Coranus siva and Brassivola hystrix (Ambrose, 1999) and was favourably biased towards the males in Sycanus reclinatus (Vennison and Ambrose, 1992).

The stadial durations of the I, II, III, IV, V instars fed on Corcyra cephalonica were 13.47 ± 0.44, 11.56 ± 0.34, 16.24 ± 0.77, 15.24 ± 0.96 and 18.57 ± 2.53 respectively (Table 1). The total stadial period from egg to adult was 75.08 with the fifth larval stage being the longest. The time needed to complete the development of one generation was 144 ± 9.45 days. The survival rates of I, II, III, IV, V instars of the predator are 85.71%, 78.57%, 60.71%, 44.64% and 12.5% respectively. The overall survival rate is 12.00% (Table 2).

The stadial durations of the I, II, III, IV, V instars fed on artificial diet were 15.76 ± 1.36, 17.00 ± 3.27, 24.25 ± 5.08, 15.00 ± 0.01 and 17.02 ± 2.56 respectively (Table 3). The total stadial period from egg to adult was 89.03 with the third larval stage being the longest. The time needed to complete the development of one generation was 155.45 ± 3.85 days. The survival rate of the I, II, III, IV, V instars of the predator are 70.00%, 53.33%, 42.36%, 23.33% and 16.36% respectively. The overall survival rate is 16.03% (Table 4).

The stadial durations of the I, II, III, IV, V instars fed on Spodoptera litura were 13.67 ± 0.55, 11.97 ± 0.60, 15.86 ± 1.04, 15.36 ± 1.47 and 16.25 ± 1.82 respectively (Table 5). The total stadial period from egg to adult was 73.11 with the fifth larval stage being the longest. The time needed to complete the development of one generation was 141.99 ± 4.45 days. The survival rate of the first, second, third, fourth and fifth instars of the predator are 78%, 60%, 44%, 22% and 15.30% respectively. The overall survival rate is 15% (Table 6).

The incubation period was minimum in the predatory reduviid, Panthous bimaculatus that were fed on artificial diet. This could ensure a faster process of multiplication of the predators. Moreover, the insects fed on artificial diet were found to be more active and energetic. This could possibly be because the energy spent in capturing the prey, in this case, the cotton ball dipped in artificial diet, is nil and the predator doesn't have to struggle to subdue the prey with its venomous saliva before it can feed on it. At the same time, there is a slight possibility that a predator which has been fed only by artificial diet, when released into the field may not be as effective as the ones fed with other smaller insects, since it has limited experience in killing and pinning down the prey with its venomous saliva. This claim is yet to be tested and confirmed. From the study it can be concluded that the predator thrives well on artificial diet in comparison to Corcyra cephalonica and Spodoptera litura. These findings could provide an insight into the optimal conditions and feed required for the successful multiplication of these predators for dissemination in pest infested fields as a part of biological control of harmful insect pest species.

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References

Ambrose, D.P. 1999. Assassin bugs. New Hampshire, USA: Science publishers and New Delhi, India: Oxford and IBH Publishing Co. Pvt. Ltd. 337 pp.

Ambrose, D.P., Rajan, S.J., Nagarajan, K., Singh, V.J. and Krishnan, S.S. 2009. Biology, behaviour and functional response of Sphedanolestes variabilis Distant (Insecta: Hemiptera: Reduviidae: Harpactorinae), a potential predator of lepidopteran pests. Entomologica Croatia 13(2): 33-44.

De Clercq, P. and Degheele, D. 1992. A meat based diet for rearing the predatory stink bugs Podisus maculiventris and Podisus sagitta (Het.: Pentatomidae). Entomophaga 37(1): 149-157.

Edwards, J.S. 1961. The action and composition of the saliva of an assassin bug Platymeris rhadamanthus Gaerst. (Hemiptera, Reduviidae). The Journal of Experimental Biology 38: 61-77.

Hwang, W.S. and Weirauch, C. 2012. Evolutionary History of Assassin Bugs (Insecta: Hemiptera: Reduviidae): Insights from Divergence Dating and Ancestral State Reconstruction. PLoS ONE 7(9): e45523. doi:10.1371/journal.pone.0045523.

Louis, D. 1974. Biology of Reduviidae of cocoa farms in Ghana. American Midland Naturalist 91: 68-89. doi:10.1093/biostatistics/4.2.249.

Miller, N.C.E. 1953. Notes on the biology of the Reduviidae of Southern Rhodesia. Transactions of the Zoological Society of London 27: 541-672. doi: 10.1093/biostatistics/4.2.249.

Miller, N.C.E. 1959. A new subfamily, new genera and new species of Reduviidae (Hemiptera-Heteroptera). Bulletin of the British Museum (Natural History) Entomology 8: 49-117.

Readio, P.A. 1927. Studies on the biology of the Reduviidae of America north of Mexico. Kansas University science bulletin 17: 1-291.

Ryckman, R.E. 1954. Reduvius senilus Van Duzee from the lodges of Neotoma in San Juan county, Utah (Hemiptera:Reduviidae). Bulletin of the Southern California Academy of Sciences 53: 88.

Sahayaraj, K. and Paulraj, M.G. 2001. Rearing and life table of reduviid predator Rhynocoris marginatus Fab. (Het., Reduviidae) on Spodoptera litura Fab.(Lep.,Noctuidae) larvae. Journal of Applied Entomology 125: 321-325. doi: 10.1046/j.1439-0418.2001.00547.x.

Schaefer, C.W. and Ahmad I. 1987. Parasites and predators of Pyrrhocoridae (Hemiptera) and possible control of cotton stainers Phonoctonus spp., (Hemipetra; Reduviidae) Entomophaga 32: 269-275.

Subramanian, K. and Kitherian, S. 2012. Survey of Reduviids in Cotton Agro-Ecosystem of Tamil Nadu, India. Middle-East Journal of Scientific Research 12(9): 1216-1223.

Swadener, S.O. and Yonke, T.R. 1973. Immature stages and biology of Sinea complexa with notes on four additional reduviids (Hemiptera: Reduviidae). Journal of the Kansas Entomological Society 6(1): 123-132.

Vennison, S.J. and Ambrose, D.P. 1992. Biology, Behaviour and Biocontrol Efficiency of a Reduviid Predator, Sycanus reclinatus Dohrn (Heteroptera: Reduviidae) from Southern India. Mitteilungen aus dem Museum für Naturkunde in Berlin. Zoologisches Museum und Institut für Spezielle Zoologie (Berlin) 68: 143–156. doi: 10.1002/mmnz.19920680110.

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New taxa of the genus Bareogonalos from Asia with further information on the tribe Nomadinini (Hymenoptera, Trigonalidae)

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Abstract

A new subgenus of the nomadinine genus Bareogonalos (Trigonalidae) is described with B. huisuni Yamane et Yamane as the type species. A trigonalid obtained from a Provespa nest is described as B. (Makotogonalos) provespae sp. n. Further information on B. (Bareogonalos) jezoensis (Uchida) is given for distribution and behavior. A map is given showing the geographical distribution of B. jezoensis. Morphological characters important for the classification of Nomadinini are discussed.

Keywords: Trigonalidae, Nomadinini, Bareogonalos, new subgenus, new species, distribution, morphological characters.

Introduction

The wasps of the family Trigonalidae (=Trigonalyidae) are distributed almost over the world, but most of them are rare insects probably reflecting their peculiar habits. They generally have intermediate hosts (lepidopteran and symphytan larvae) before reaching the final hosts (mainly hymenopterous parasitoids and social wasps). The taxonomy of this interesting family was initiated by Schulz (1907) and Bischoff (1933, 1938), and the current classification system was established by Carmean and Kimsey (1998), who reduced the number of subfamilies from six (Weinstein and Austin, 1991) to one, also combining several genera into fewer groups, while erecting a new subfamily and new genus. Although Popov (1945) suggested that the ancient hosts of the family were Vespidae rather than Ichneumonidae, Carmean's and Kimsey's (1998) phylogenetic analysis shows a reversed scenario.

The species of the trigonaline genus Bareogonalos parasitize the nest of vespid social wasps of the subfamily Vespinae. All the described species of this genus have been known from nests of the genera Vespa, Vespula, Dolichovespula and/or Provespa (Carmean, 1991; Carmean and Kimsey, 1998; Matsuura and Yamane, 1990; Yamane, 1973; Yamane and Yamane, 1975; Weinstein and Austin, 1991). In this paper a new species of Bareogonalos is described that was reared from a nest of Provespa in Sumatra, Indonesia (Matsuura and Yamane, 1984). A new subgenus is established for two species, B. huisuni Yamane et Yamane and B. provespae sp. n., mainly based on the mandibular structure and palpal formula. Other information on the distribution and biology of B. jezoensis (Uchida) and a discussion on important morphological characters in the tribe Nomadinini are also given.

Materials and Methods

The holotype of Bareogonalos huisuni Yamane et Yamane was borrowed from the Entomological Collection of the Hokkaido University Museum (SEHU). The other material is now at the SKY Collection in Kagoshima, but will be deposited in SEHU in the near future. The holotype of B. provespae will be deposited in the Entomological Collection of Museum Zoologicum Bogoriense, Indonesia. Morphological characters were observed with a Nikon Stereomicroscope SMZ18. Information on the distribution and biology of B. jezoensis was

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collected through previous literature, internet websites and with the help of my friends.

Taxonomy

Genus Bareogonalos Schulz

Bareogonalos Schulz, 1907: 18. Type species: Trigonalys canadensis Harrington, 1896; Carmean and Kimsey, 1998: 60.
Nippogonalos Uchida, 1929: 79. Type species: N. jezoensis Uchida, 1929 by monotypy; Tsuneki, 1991: 4; Lelej, 1995: 12. Synonymized with Bareogonalos by Bischoff, 1938: 14.

Diagnosis. Antenna slender, forward-directed, with 19-23 segments in male and 19-21 segments in female; scape and pedicel smooth or very superficially punctate and shiny. Median furrow on frons indistinct. Mandible 'schnabelform'; mandibles symmetrical or asymmetrical; right mandible with 4 teeth, left mandible with 3 or 4 teeth. Maxillary palpus with 5-6 segments; labial palpus with 3 segments. Eye normally positioned; its lower margin at the level above mandibular base. Deep depression behind ocelli absent. Pronotum normal, not overhanging anteriorly; its lower lateral lobe more or less incised. Parapsidal line on mesoscutum carinate, not excavated as in Bakeronymus and Pseudonomadina. Metanotum flat or pyramidal. Apical spur of foretibia shallowly bifid apically (in male smaller branch sometimes rudimentary). Gastral tergites with minute to small punctures, smooth or mat; female gaster thick, elongate-globular; its sternites 2 and 3 armed with posteriorly directed processes/lobes (armature sensu Carmean and Kimsey, 1998). Hind trochanter 2-segmented. Second and third cubital cells clearly separated.

All the known species have been collected from nests of vespine species (van der Vecht, 1933; Yamane, 1973; Yamane and Yamane, 1975).

Makotogonalos Yamane, subgen. nov.

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Type species: Bareogonalos huisuni Yamane et Yamane, 1975.
Diagnosis. Antenna with 20-23 segments in male and 19-21 segments in female. Mandibles symmetrical; both right and left mandibles with 4 teeth (Fig.1); basal tooth bifid apically. Maxillary palpus 5-segmented; labial palpus 3-segmented; terminal segment of labial palpus not modified (Figs. 2, 3). Anterior lateral lobe of pronotum distinctly incised below. Metanotum normal, not distinctly raised. Punctation on gastral tergites small but distinct and dense; tergites mat, only weakly shiny. Sexual difference not strongly pronounced; male gaster weakly tapered posteriorly, with similar maculation to that of female.

Etymology. The subgeneric name is composed of 'Makoto' and 'gonalos', the latter having been frequently used as a suffix for trigonalid genera. 'Makoto' is the given name of the collector of the new Sumatran species, the late Dr. Makoto Matsuura, its meaning being 'true' or 'faithfulness'.

The nominal subgenus Bareogonalos is characterized as follows: antenna 19-23 (usually 21) segments in male and 20-21 (usually 21) in female. Mandibles asymmetrical; right mandible with 4 teeth, left mandible with 3 teeth. Maxillary palpus 6-segmented; labial palpus 3-segmented; terminal segment of labial palpus strongly flattened, more or less spatulate (Fig. 4). Lower lateral lobe of pronotum only weakly incised. Metanotum pyramidal. Punctation on gastral tergites minute; tergites nearly smooth and very shiny. Sexual difference more pronounced (in at least B. jezoensis); male gaster distinctly tapered posteriorly, with more or less reduced pale maculation.

Two species are included in the new subgenus, namely Bareogonalos huisuni Yamane et Yamane from Taiwan, and B. provespae Yamane, sp. n. from Sumatra, Indonesia.

Bareogonalos huisuni Yamane et Yamane (Figs. 1, 2, 6-9)

Bareogonalos huisuni Yamane et Yamane, 1975: 456. Type locality: Kwantau-Shih, Taiwan; Carmean and Kimsey, 1998: 61; Chen et al., 2014: 18 (redescription).

Female.

Measurements (in mm). Paratype from Vespula flaviceps karenkona nest: body length 11.1; head width including eyes 2.96; eye length 1.23; interocular distance at the level of anterior

ocellus 1.96; distance between eye and posterior ocellus 0.75; distance between outer margins of posterior ocelli 0.73; scape length 0.43; mesonotal width just before tegulae 2.80; hind tibial length 2.68; forewing length 10.7. Specimens from Vespa velutina flavitarsus nests (n=5): body length 12.9-15.1 (mean: 14.8); head width 3.52-3.88 (3.75); interocular distance 2.28-2.60 (2.48); distance between eye and posterior ocellus 0.80-0.90 (0.88); distance between outer margins of posterior ocelli 0.83-0.98 (0.90); scape length 0.50-0.53 (0.52); mesonotal width 3.44-3.92 (3.78); hind tibial length 3.04-3.48 (3.31); forewing length 12.3-14.3 (13.8).

Description. (Larger specimens reared from Vespa velutina flavitarsus). Head distinctly broader than long; inner margins of eyes weakly convergent below. Clypeus transverse, with apical margin weakly emarginate medially and basal margin straight. Apical to third mandibular teeth bluntly pointed apically; basal tooth very broad at base and bifid shallowly at apex. Distance between outer margins of lateral ocelli almost as long as that between eye and posterior ocellus. Pair of small but distinct pits present behind ocelli (pits not connected by a narrow furrow); area behind ocelli not raised. Antenna with 19-20 segments. Median sulcus of mesoscutum located in a broader furrow margined laterally by low carinae that are less than half the mesoscutum length, not very clearly separated from rugoso-reticulum on its both sides, but complete, reaching the posterior margin of scutum; notaulix complete, its bottom with transverse carinae; parapsidal line carinate; notaulix and parapsidal line more parallel than in B. provespae; mesoscutellar disc roundly raised, with pair of distinct posterolateral tubercles; dorsal face of mesoscutellum gradually merging into posterior face. Metanotum demarcated from mesoscutellum and propodeum by deep sulci; its disc reversed-trapezoidal with flat dorsal surface, elevated from level of lateral parts of metanotum. Propodeum with same inclination as metanotum; dorsal face weakly produced laterally. First gastral tergite small and almost vertical; second tergite largest. Second and third sternites each apically with median process; that on second sternite broader than in B. provespae and medially weakly emarginate; that on third sternite short.

Head including clypeus very minutely punctate, and moderately shiny; supra-antennal area irregularly sculptured (often transversely striate) and mat. Lateral face of pronotum coarsely rugose; dorsa of mesoscutum and mesoscutellum coarsely reticulate; lower half of mesopleuron weakly punctate and moderately shiny; posterior slope of mesoscutellum finely sculptured, mat. Median disc of metanotum finely sculptured, mat; lateral portion of metanotum rugose with large punctures along posterior margin but interspaces shiny. Propodeum coarsely reticulate; its ventrolateral portion with 2-3 strong carinae. Second and subsequent gastral tergites very finely and densely punctate, only slightly shiny; lateral portion of gaster, first tergite and sternites more shiny. Femora smooth and shiny, other parts of legs finely and densely sculptured and mat. Whole body with dense erect to suberect hairs; those on gastral tergites shorter than those on other parts of body; maxillary and labial palpi with strong erect hairs.

Body black, with the following parts yellowish to orange-yellow: antennal scape; upper lateral portion of pronotum close to tegula; pair of elongate cuneiform markings on mesonotum anteriorly; pair of smaller markings adjacent to notaulices; entire mesoscutellar disc and axilla largely; transverse line on metanotum (often interrupted medially); median spot (often lost) and pair of large lateral spots on propodeum; basal bands on second to fifth gastral segments, those on fourth and fifth with median extension toward their apical margin, small spot on sixth tergite; apical process of second sternite (often enlarged behind the lobe); upper face of hind coxa and hind trochanter entirely. Mandible and antenna (at least basal segments) reddish brown. Wings yellowish brown, particularly darker in anterior 2/3 of forewing; forewing with dark area near apex.

Male.

Measuremets (in mm). Holotype from Vespula flaviceps karenkona nest: body length 11.0; head width including eyes 3.32; eye length 1.35; interocular distance at the level of anterior ocellus 2.16; distance between eye and posterior ocellus 0.80; distance between outer margins of posterior ocelli 0.73; scape length 0.48;

mesonotal width just before tegulae 3.08; hind tibial length 2.72; forewing length 10.6. Larger specimens reared from Vespa velutina flavitarsus (n=5): body length 12.9-15.0 (mean 13.7); head width including eyes 3.48-3.84 (3.60); eye length 1.4-1.53 (1.46); interocular distance at the level of anterior ocellus 2.24-2.52 (2.35); distance between eye and posterior ocellus 0.75-0.85 (0.79); distance between outer margins of posterior ocelli 0.83-0.88 (0.86); scape length 0.53-0.55 (0.54); mesonotal width just before tegulae 3.36-3.84 (3.58); hind tibial length 2.84-3.24 (3.04); forewing length 11.1-12.1 (11.7).

Description. (Larger specimens reared from Vespa velutina flavitarsus). Structure, sculpture and coloration similar to those of female. Antenna with 20-21 segments, in total length much longer than in the female. Distance between outer margins of lateral ocelli slightly longer than that between eye and posterior ocellus. Gaster narrowed both anteriad and posteriad; second and third sternites without apical processes. Fourth to seventh tergites extensively or entirely yellow. Body markings much reduced in some specimens.

Specimens examined (except for the holotype and paratypes): 1♀, Shanlinchi, Nantou Hsien, 16 ix 1995; 1♂, Kuantaushan, Lenai, Nantou Hsien, 23 ix 1996; 1♂, Sungkang, Nantou Hsien, Taiwan, 15 iv 1996; 1♀, Fenglin, Nantou Hsien, 13 ix 1997; 11♂9♀, Nanshanchi, Nantou Hsien, 13-19 xi 1997 (reared from a nest of Vespa velutina flavitarsus Sonan; all specimens collected by C.C. Luo).

Remarks. This species was originally described based on the specimens obtained from Vespula flaviceps karenkona Sonan nests in Taiwan (Yamane and Yamane, 1975). These specimens are naturally small in size as in the specimens of B. jezoensis collected from Vespula and Dolichovesoula nests in Japan. In 1985 additional specimens were collected by Mr. C. C. Luo (Puli, Taiwan) also in Taiwan. They are comparable in size to the specimens of B. jezoensis from Vespa species in Japan. The larger and smaller specimens of B. huisuni differ from each other only in size; distinct differences have not been detected in basic structure and coloration between them.

Chen et al. (2013) stated that the illustrated paratype in Yamane and Yamane (1995) is a female not a male as reported by Yamane and Yamane (1975), and that the male is unknown (they even changed the sex of the holotype from the male to female, surprisingly, without examining it). Their view is very strange, because sexual differences are clearly mentioned in the original description. The illustrated paratype (Fig. 2 in Yamane and Yamane, 1975) is, of course, a female as was mentioned by the authors, who never said that the specimen is a male. The holotype is a male (Fig. 1), which was examined again this time. Probably Chen et al. (2013) did not read the original description at all, or were unable to recognize differences between the sexes. The male and female of this species are very easily distinguished based on the characters mentioned in the original description and redescription given above. Even just a glance at Fig. 1 (male) and Fig. 2 (female) in the original description is enough to identify the sex.

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Bareogonalos provespae Yamane, sp. n.
(Figs. 3, 10-13)

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Female (paratype).
Measurements (in mm). Body length 13.4; head width including eyes 3.52; eye length 1.43; interocular distance at the level of anterior ocellus 2.44; distance between eye and posterior ocellus 0.83; distance between outer margins of posterior ocelli 1.00; scape length 0.50; mesonotal width just before tegulae 3.56; hind tibial length 3.20; forewing length 12.0.

Description. Head distinctly broader than long; inner margins of eyes weakly convergent below. Clypeus transverse, with apical margin more deeply emarginate medially than in B. huisuni and basal margin weakly emarginate. Apical to third mandibular teeth pointed apically; basal tooth very broad at base and bifid shallowly at apex. Distance between outer margins of posterior ocelli distinctly longer than that between eye and lateral ocellus. Pair of small pits behind posterior ocelli indistinct; the pits

connected by a narrow groove; area behind lateral ocelli raised to form square platform. Antenna with 21 segments. Median sulcus of mesonotum obsolete, located in broader furrow that reaches midlength of mesonotum and is margined laterally by carinae; notaulix complete, its bottom with transverse carinae; parapsidal carina forming triangular area together with notaulix, opened anteriorly; mesoscutellum only feebly convex dorsad, without distinct posterior tubercles; dorsal face of mesoscutellum demarcated from steeper posterior face only by sculpturation. Metanotal disc transversely rectangular with flat dorsal surface, elevated from lateral parts of metanotum. Propodeum with same inclination as metanotal disc and mesoscutellum, with lateral triangular area that is depressed. First gastral tergite small and steep; second tergite largest. Second sternite apically with median process that has round apex; that on third sternite shorter.

Head very minutely and densely punctate, weakly shiny; mandible, vertex, temple and gena more strongly shiny. Lateral face of pronotum coarsely rugose; dorsa of mesoscutum and mesoscutellum coarsely reticulate; lower half of mesopleuron and metapleuron smooth to weakly sculptured; posterior slope of mesoscutellum finely sculptured. Median disc of metanotum finely sculptured, mat; lateral portion of metanotum nearly smooth with large punctures along posterior margin. Propodeum very coarsely rugoso-reticulate. Gastral tergites finely and densely punctate and mat, but lateral portion and sternites more shiny. Femora rather smooth and shiny; other parts of legs finely and densely sculptured and mat. Whole body with dense erect to suberect hairs; those on gastral tergites shorter than those on other parts of body; maxillary and labial palpi with strong erect hairs.

Body black, with the following parts yellowish or orange-yellow: posterior face of propodeum, first gastral segment, second tergite basally, second sternite except for lateral portion, upper face of hind coxa and hind trochanter entirely. Mandible and antennal flagellum reddish brown. Anterior 2/3 of forewing yellowish brown. The single female specimen is somewhat teneral so that the lighter body portions might be slightly darker in maturation.

Male (holotype and paratype).
Head of the paratype missing.
Measurements (in mm; n=1). Body length 12.3; head width including eyes 3.44; eye length 1.48; interocular distance at the level of anterior ocellus 2.20; distance between eye and posterior ocellus 0.78; distance between outer margins of posterior ocelli 0.90; scape length 0.50; mesonotal width just before tegulae 3.40; hind tibial length 3.00; forewing length 10.9.

Description. Structure, sculpture and coloration similar to those of female. Antenna with 22-23 segments. Caphalic pits behind ocelli very obsolete. Gaster distinctly narrowed both anteriad and posteriad; second and third gastral sternites without armature (processes).
Holotype: male, collected from a Provespa nocturna Vecht nest (No. 8101), 15.xi.1981, Kotabumi, West Sumatra, Indonesia, M. Matsuura leg. (MZB, Indonesia). Paratypes: 1 male, same data as above, but emerged on 22.xi.1981; 1 female, same data as holotype.

Etymology. The specific epithet is named after the host vespid Provespa.

Remarks. It was rather unexpected that the two species of the subgenus Makotogonalos use very different groups of Vespinae as their hosts (Vespa and Vespula by B. huisuni, and Provespa by B. provespae). Furthermore, the other Indonesian species B. jezoensis known from Java belongs to the other subgenus (Bareogonalos). The latter species is originally recorded from temperate Japan (Uchida, 1929; Yamane, 1973) and Russian Far East (Lelej, 2003), located very far from Java, but the two populations use Vespa species as hosts; the other hosts for the Japanese population, i.e. Vespula and Dolichovespula, are absent from Java. With very scanty information I cannot say with certainty that in Indonesia the two Bareogonalos species overlap in both their hosts and distribution, but this may be possible. On the other hand, Carmean (1991) suggested the possibility that B. jezoensis had been introduced to Java with ornamental plants carrying trigonalid eggs deposited in their foliage, because no locality had been known between

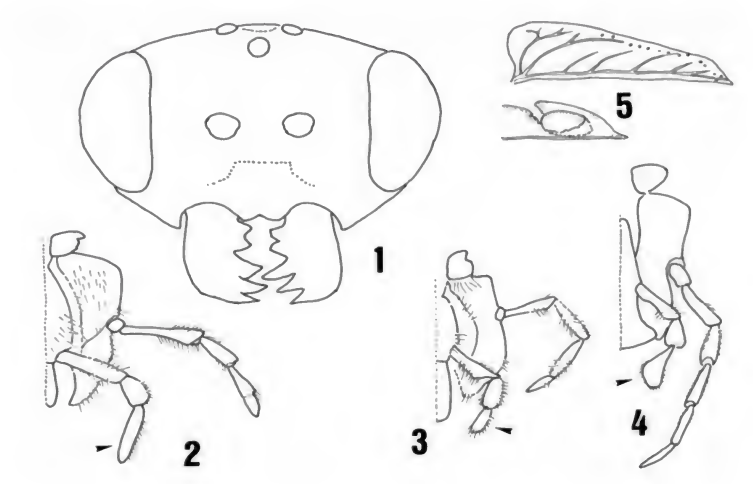
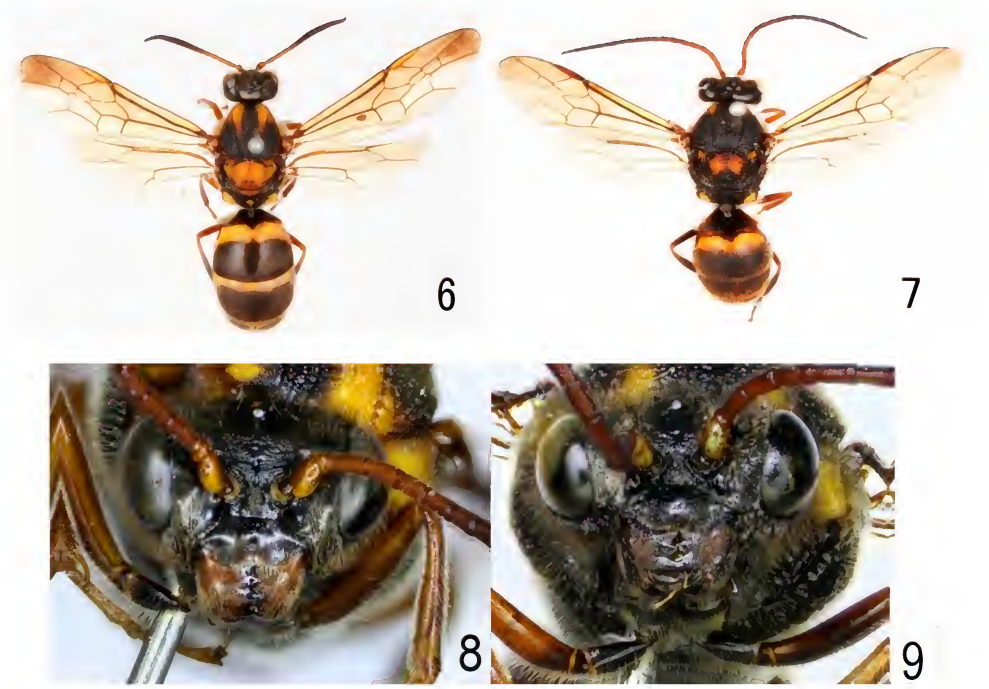


Fig. 1. Head of Bareogonalos provespae sp. n. (male) in frontal view. 2. Maxillary and labial pulpi of same specimen in ventral view. 3. Same, B. huisuni (male). 4. Same, B. jezoensis (male). 5. Mimosa leaf receiving B. jezoensis eggs (above) and an egg (below) (redrawn from Nozaka, 1976 with permission).



Figs. 6-9. Bareogonalos huisuni (ex. Vespa velutina nest). 6, 8. Female. 7, 9. Male.

Figs. 10-13. Bareogonalos provespae. 10, 12. Female. 11, 13. Male.

Japan and Java, and because the collection site in Java was a tourist site.

Additional information on Bareogonalos (Bareogonalos) jezoensis (Uchida)

Distribution. After the publication of Yamane (1973) many localities have been added to the distribution of this species (Fig. 14):
Primori'e (Russian Far East). Kamenushka, Ussuriysk Reserve (Lelej, 2003); Vysokogsk (A.S. Lelej, pers. comm., 5 Oct. 2014).
Hokkaido. Hamamasu and Atsuta, Ishikari-shi (Matsuura, 1995); Otaru, ix.1984, T. Ban leg., reared from Vespa simillima nest; Akanuma, Assabu-cho (Akihide Nomura: arisuabu.exblog.jp, 15.x.2014; Fig. 15).
Honshu. Fukushima Pref.: Kohri-machi, Date-gun (Ono, 1988); Kurotanikawa, Tadami, 14.ix.1963, M. Kanno. Niigata Pref.: Muroya, Aga-machi; Yagisawa and Kaigake Spa, Yuzawa-machi (Itami, 2011); Yunotani and Maruyama, Uonuma-shi (Iwata and Fukada, 2010). Nagano Pref.: Tokuhara (Tsuneki, 1991); Seinaiji-mura, Shimoina-gun (Ono, 1987); 2 males & 3 females, Hakuba, 12.x.1990, reared from V. simillima nest; 1 male, Tokisato, Iiyama-shi, Nagano Pref., 18–24.viii.1998, A. Shimizu (Shimizu Collection). Toyama Pref.: Arimine, Toyama-shi (Matsuura, 1991). Fukui Pref.: Ohno-shi (Nozaka, 1976); 1 male, Hatogayu, 26-30.ix.1982, T. Tano leg.; 1 female, Satsura, Izumi-mura, 18.ix.1982, T. Tano leg.; 1 female, Taniyama, 5.x.1972, T. Tano leg.; 1 male, Arashi, 23.ix.1977, H. Kurokawa leg. Ishikawa Pref.: Hakusan (Nakamura, 1978).
Kyushu. Miyazaki Pref.: Fudono, Shiiba-son, Higashiusuki-gun (from a Vespa simillima nest; 1 female, photograph taken on 10 Oct. 2003; N. Nasu, pers. comm., 13 Oct., 2014).

In Northeastern Asia this species occurs in Primori'e (Russion Far East), Hokkaido, Honshu and Kyushu, with the southernmost locality in a mountain range in Kyushu (Shiiba-son,

Miyazaki Pref.). Shikoku apparently lacks it. Up to now no record of this species is available in the Korean Peninsula (J. K. Kim, personal comm., 13 Oct. 2014).

Biological notes. An Australian species, Taeniogonlos maculata (F. Smith), and some others are known to be primary parasites of sawflies and probably of lepidopteran larvae (Carmean and Kimsey, 1998; Raff, 1934). Other species are parasitoids of parasitic wasps and tachinid flies that attack larvae of lepidopterans and sawflies (Clausen, 1940). Not a few species, however, have been found from nests of solitary and social wasps such as eumenids, polistines and vespines (Bischoff, 1938; Iwata, 1971; Carmean, 1991; Weinstein and Austin, 1996). Among them are Licogaster, Xanthogonalos, Seminota (S. America), Bakeronyms, Nomadina, Pseudonomadina (S. America and SE Asia), and Boreogonalos (N. and C. Americas, and E. Asia).

The life cycle of Bareogonalos jezoensis was briefly discussed by Yamane (1973) with reference to Clausen's (1931) observations on Poecilogonalos maga Teranishi (currently Taeniogonalos maga). Probably first instar larvae are carried to vespine nests in food pellets made of parasitized caterpillars that are captured and chewed by vespine workers. After entering a host wasp larva (or prepupa) planidium first instar larvae, though not yet observed actually, probably moult into second instar larvae that might moult into third instar shortly. Third instar larvae are of mandibulate type, fighting with each other leaving only one individual in the host. The survived fourth instar larva grows inside the host and come out to host's body surface and moults into a fifth (final) instar larva that consumes the host from outside (Yamane, 1973).

Adult trigonalids are collected in the field and from vespine nests during June to October in temperate Japan (Yamane, 1973; Tsuneki, 1973, 1991; Nozaka, 1976; Iwata and Fukada, 2010). Some males were collected in early June when prepupae are generally not available in the nests of vespines in Hokkaido. Yamane (1973) suggested the possibility that this species overwinters as adults. However, later observations made by Nozaka (1976) disagree with this view because the oviposition was conducted during October to leaves of the mimosa Albizia julibrissin Durazz. (Fabaceae) native to Japan (Fig. 5). Tsuneki (1973) listed other deciduous trees such as willows,

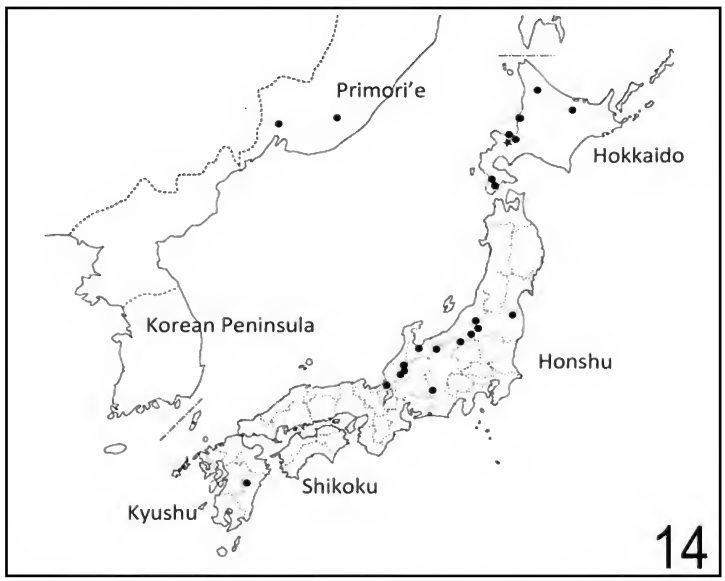


Fig. 14. Distribution of Bareogonalos jezoensis in Far Eastern Asia. ★ indicates the type locality (Jozankei, Sapporo, Hokkaido)

Table 1. Hosts of Bareogonalos species in Asia

B. jezoensis	Vespa analis analis	Vecht 1933 (West Java)
	Vespa analis insularis	Haneda 1993 (Honshu), Sayama 1999 (Hokkaido)
	Vespa crabro flavofasciata	Yamane 1973 (Hokkaido), Sayama unpubl. (Hokkaido)
	Vespa dybowskii	Matsuura 1995 (Hokkaido), Sayama unpubl. (Hokkaido)
	Vespa mandarinia japonica	Ono 1988 (Honshu)
	Vespa simillima simillima	Yamane 1973 (Hokkaido)
	Vespa simillima xanthoptera	Ono 1987 (Honshu), Iwata and Fukada 2010 (Honshu), this paper (Kyushu)
	Vespa velutina velutina	Vecht 1933 (West Java)
	Vespula shidai	Yamane 1973 (Hokkaido; as Vespula sp.)
	Vespula vulgaris	Yamane 1973 (Hokkaido)
	Vespula schrenckii	Yamane 1973 (Hokkaido; as V. rufa)
	Dolichovespula media media	Sayama unpubl. (Hokkaido)
	Dolichovespula media sugare	Matsuura 1995 (Honshu)
	Dolichovespula saxonica nipponica	Yamane 1973 (Hokkaido; as D. pacifica), Makino 1982 (Hokkaido), Sayama (unpubl., Hokkaido)
B. huisunu	Vespa velutina flavitarsus	This paper (Taiwan)
	Vespula flaviceps karenkona	Yamane & Yamane 1975 (Taiwan)
B. provespae	Provespa nocturna	Matsuura & Yamane 1990 (West Sumatra)

Fig. 15. A Bareogonalos jezoensis female ovipositing on a leaf of Atremisia montana (6 Oct. 2013, Assabu-cho, Hokkaido, Japan; photo by Akihide Nomura; arisuabu.exblog.jp 15 Oct. 2014). 16. A teneral female of the same species collected from a Vespa simillima comb cell (18 Oct. 2003, Shiiba-son, Miyazaki Pref., Kyushu; photo by Naoko Nasu).

walnuts, alders etc., in which oviposition behavior was confirmed. Furthermore, overwintering adult trigonalids have never been found in Japan.

The oviposition found during autumn poses another problem. Albizia julibrissin is a deciduous tree, shedding all the leaves before winter so that all the eggs laid in the leaves would not survive. An important hint was presented for the North American congener B. canadensis by Carmean (1988, 1991). He studied the biology of B. canadensis in Oregon, USA, where the oviposition was seen in some plants and even paper under laboratory condition. Among the presented plants female wasps laid eggs more frequently in the leaves of Douglas-firs (Pseudotsuga menziessi) and Western hemlocks (Tsuga heterophylla), both being conifers with evergreen leaves. These eggs can survive on the trees until next spring. On the other hand, all the trees used for the oviposition by B. jazoensis are deciduous (Carmean's citation of bamboo and fern from Tsuneki, 1973, is not correct). I strongly suggest that lepidopteran larvae, or even leaf litter arthropods, which feed on live or fallen leaves during autumn and having overwintered, and those feeding fallen leaves next spring are candidates for the intermediate hosts of this species.

Several final host species have been found after Yamane (1973). All the host species are listed in Table 1.

Important characters in the classification of the nomadinine genera

In the course of taxonomic studies of social wasps of the Old World I have paid attention to their enemies especially of the family Trigonalidea (e.g., Yamane, 1973; Yamane and Terayama, 1983); some new taxa have been described in the tribe Nomadinini and their hosts revealed. It has become evident that some species have unusual numbers of mandibular teeth, and palpal segments of maxilla and labium; reduction in wing venation, thickening of antennae, etc. have also been noticed. Although the biological significance of these characters is not known, these characters seem to be important in the classification of the tribe Nomadinini (sensu Carmean and Kimsey, 1998).

Groups discussed.

1. Genus Licogaster Shuckard, 1841 (3 spp.)
2. Genus Xanthogonalos Schulz, 1907 (1 sp.)
3. Genus Seminota Spinola, 1840 (5 species in the world)
4. Genus Nomadina Westwood, 1868 (4 spp.)
5. Genus Pseudonomadina Sk. Yamane et Kojima, 1982 (1 sp.)
6. Genus Bakeronymus Rohwer, 1922 (2 spp.)
7. Genus Bareogonalos Schulz, 1907 (4 spp.)

The characters were observed with dry specimens or cited from previous literature. Although Carmean and Kimsey (1998) listed only one species for Bakeronymus, B. typecus seidakka Yamane et Terayama was raised to full species (Chen et al., 2014).

1. Mandibular shape. Schulz (1907) mentioned that in the 'Nomadinae' and 'Bareogonalinae' the mandibles are much constricted and produced downward (schnabelform) like a beak. This condition is peculiar to these wasps and no doubt derived, though in Bareogonalos the condition is less distinct than in Bakeronymus, Nomadina and Pseudonomadina.

2. Number of mandibular teeth. Although Schulz (1907) counted the asymmetrical mandibles (right with 4 teeth, left with 3) as a characteristic defining the family, symmetrical mandibles occur in Nomadinini. In Bakeronymus and Makotogonalos (subgenus of Bareogonalos) (these taxa were not known to Schulz) both the mandibles have four teeth, and in Pseudonomadina (also not known to Schulz) both have three. I have not examined specimens of any species of Nomadina, but Westwood (1868) in his original description of the genus wrote "mandibulae 4-dentatae", and Carmean (1995) states that the mandibles are symmetrical. I consider the asymmetrical mandibles to be the ground plan for Trigonalidae, the symmetrical ones being regained within this family (see also Carmean and Kimsey, 1998).

3. Vertical furrow on frons. In Bakeronymus and Pseudonomadina the frons has a broad vertical furrow from the upper margin of clypeus to just below the anterior ocellus. Schulz's (1907) description of Nomadina cisandina (Schulz, 1905) and Bischoff's (1933) description of

N. nasuta Bischoff, 1933 (=N. balteata (Cameron, 1899)) suggest that this condition also occurs in at least some species of Nomadina. This character state is peculiar to Bakeronymus, Nomadina and Pseudonomadina and no doubt represents a derived condition.

4. Clypeal shape. In Bakeronymus and Pseudonomadina the clypeus is completely flat, continued from the supraclypeal area that is part of the frontal furrow. This condition very probably occurs also in Nomadina. In Seminota, Xanthogonalos and Bareogonalos, the clypeus is raised, seen in profile higher than the supraclypeal area. The flat clypeus uniquely occurs in Bakeronymus, Nomadina and Pseudonomadina, and is considered to be derived.

5. Position of eyes. In Bakeronymus and Pseudonomadina the eyes are very large, and the lower margin of the eye is below the level of the mandibular base; the oculo-malar space is quite short. This condition is only found in these genera among Trigonalidae, and seems derived.

6. Deep depression behind ocelli. Vertex behind ocelli is generally flat or roundly convex in this tribe. Only in Bakeronymus and Pseudonomadina this area has a deep depression (Yamane and Kojima, 1982); this state may be derived.

7. Antennal shape. In most of the genera treated here the antennae are slender; flagellar segments are rarely moniliform (beadlike). In Bakeronymus, Nomadina and Pseudonomadina the antennae are thick (especially in the female) and relatively short; the segments are often strongly moniliform (see Fig 3 in Yamane and Kojima, 1982). The moniliform flagellar segments are another derived condition within the Nomadinini.

8. Number of antennal segments. The number may vary even in a single species as well as within a genus (for Bareogonalos, see Yamane, 1973; Yamane and Yamane, 1975). However, generic differences are also noted. In Seminota and Xanthogonalos it is always more than 21 and in Lycogaster 22-24; in Bareogonalos it varies between 19 and 23. In Bakeronymus, Nomadina and Pseudonomadina, however, it ranges from 13 to 16. The reduced number of antennal segments represents a derived condition among the Trigonalidae.

9. Number of segments in maxillary and labial palpi. Generally in the Trigonalidae the maxillary palp has 6 and labial palp 3 segments (Schulz, 1907). Seminota, Xanthogonalos and Bareogonalos (s. str.) have the same numbers (6, 3), while in the subgenus Makotogonalos they are 5 and 3 respectively. In Bakeronymus and Pseudonomadina, the numbers are further smaller, 4 and 2 respectively; in the latter these palpi are much reduced in size. In Nomadina the maxillary and labial palpi are dwarf or even lost (Carmean, 1995; Schulz, 1907, did not mention the precise numbers of palp segments). The reduction in both the number and size of segments may be derived for the nomadinine genera.

10. Anterior part of pronotum. In some trigonalid genera the pronotum anteriorly weakly overhangs, but in many others this condition is not observed. Bakeronymus and Pseudonomadina have a strongly overhanging pronotum (seen in profile the upper portion of the anterior part strongly protruding anteriad). This is probably a derived condition.

11. Lower lateral lobe of pronotum. This part is distinctly incised in Makotogonalos and at least weakly incised in Bareogonalos (s. str.) (see Figs. 14 and 15 in Yamane and Yamane, 1975). The condition is peculiar to Bareogonalos and considered derived.

12. Shape of metanotum (postscutellum). The metanotum is flat and roundly convex in all the trigonalid genera except in Bareogonalos (s. str.), the members of which have a pyramidal metanotum with a bifid apex. Although this condition is very distinct in B. canadensis (Harrington, 1896) and B. jezoensis (Uchida, 1929), Cameron's (1897) original description of the Mexican Trigonalys scutellaris (=Bareogonalos scutellaris) leaves some doubt about the constant occurrence of this state in this species. At any rate the pyramidal metanotum is

confined to this subgenus and no doubt represents a derived condition.

13. Cubital cell of forewing. Distinction of second from third cubital cell is constant through most of the trigonalid genera, but is often absent in Nomadina and Pseudonomadina. The absence of the separation represents a derived condition.

14. Hind trochanter. In the Symphyta and some Apocrita there is an apparent second segment to the trochanter (trochantellus) that is morphologically a piece cut off the proximal end of the femur (Richards, 1977). In the Trigonalidae there often is an additional subdivision of the trochantellus so that the trochanter appears 3-segmented. The hind trochanter is '2-segmented' only in Bakeronymus, Nomadina, Pseudonomadina and Bareogonalos (Carmean and Kimsey, 1998). The frequent occurrence of the '2-segmented' hind trochanter in Ichneumonidae suggests that it represents an ancestral condition, but in Seminota and Xanthogonalos (closely related to these four genera) the hind trochanter is '3-segmented'. This means that the '2-segmented' hind trochanter in Nomadinini might be a regained condition within the Trigonalidae.

15. Apical spur on foretibia. In some nomadinine groups the apical spur of the foretibia is bifid apically; this condition is more distinct in the female. In other groups the spur is simple or missing.

16. Median processes (armature) on gastral sternites. The female trigonalids except for those of 'Trigonaloinae' have a median process either on the gastral sternite 2 or 3, or on both (Schulz, 1907). It is thought to function as an anchor for the basal part of the gaster to the upper surface of plant leaves during oviposition (Riek, 1970). In the 'Lycogastrinae' (heterogeneous group including Lycogaster) and 'Seminotinae' (Seminota + Xanthogonalos) the structure is more or less developed on both the sternite 2 and 3 (Schulz, 1907) (Xanthogonalos fasciatus Bertoni, 1912, which has the process only on sternite 3, was moved under Trigonalys by Carmean and Kimsey, 1998). In Bakeronymus, Nomadina and Pseudonomadina it is seen only on sternite 3 (Nomadina), or the process on sternite 2 is much reduced to a pair of minute spines (Bakeronymus and Pseudonomadina). In the latter two genera the process on sternite 3 is bifid apically. It is more developed on sternite 2 than on sternite 3 in Bareogonalos. The bifid process on sternite 3 and minute spinous ones on sternite 2 should represent a derived condition among the Nomadinini.

17. Punctures on gastral tergites. Punctures on gastral tergites are minute in Nomadina?, Bakeronymus, Pseudonomadina and Bareogonalos (s. str.) (in the last the gaster is smooth and very polished), or small but distinct and dense in Makotogonalos (the tergites are only weakly shiny). The fine punctation may represents a derived condition among the Nomadinini.

18. Final hosts. For Afrigonalos Carmean and Kimsey, 1998, and Xanthogonalos, nothing is known for intermediate and final hosts. Among Lycogaster ichneumonid wasps, eumenid wasps and social vespids are known as final hosts. All the species of the Neotropical genus Seminota parasitise polistine wasps (Weinstein and Austin, 1991). Final hosts are also polistines in the Neotropical genus Nomadina, and the Southeast Asian Bakeronymus and Pseudonomadina (Carmean and Kimsey, 1998; Weinstein and Austin, 1991; Yamane and Terayama, 1983). The species of Bareogonalos have been known from vespine species, but the host is not known for B. scutellaris from Mexico (Carmean and Kimsey, 1998).

The Nomadinini forms a natural group with the female having the primary armature (process/lobe) generally on gastral sternum 2 and secondary armature on sternum 3, and the male secondarily having lost tyloids on antennae (Carmean and Kimsey, 1998). Many characters analyzed above suggest that Bakeronymus, Nomadina and Pseudonomadina can be recognized as a natural group, and grouped with Bareogonalos based on the "2-segmented" hind trochanter and beak-shaped mandibles. However, Bareogonalos contains two distinct groups that can be treated as subgenera.

Table 2. Comparison of important morphological characters among some nomadinine genera.

	Nomadina	Pseudonomadina	Bakeronymus	Subgen. Bareogonalos	Subgen. Makotogonalos
Mandibular shape	Schnabelform	Schnabelform	Schnabelform	Schnabelform	Schnabelform
No. of mandibular teeth	Right: 4, Left: 4,	Right: 3, Left: 3	Right: 4, Left: 4	Right: 4, Left: 3	Right: 4, Left: 4
Vertical furrow on frons	Present?	Broad, distinct	Broad, distinct	Not distinct	Not distinct
Clypeus	Flat?; longer than broad	Flat, in profile continued from supraclypeal area; broader than long	Flat, in profile continued from supraclypeal area; broader than long	Normal; broader than long	Normal; broader than long
Position of eye	Oculo-malar space short?	Lower margin of eye below level of mandibular base	Lower margin of eye below level of mandibular base	Normal	Normal
Deep depression behind ocelli	Absent	Distinct in male and female	Distinct in male, shallow in female	Absent	Absent
Shape of antenna	Thick, short particularly in female	Thick, short	Slender	Slender	Slender
Direction of antenna	?Forward	Downward	Downward	Forward	Forward
No. of antennal segments	11-16 (generally 16) in both sexes	14 in male, 13 in female	14 in male, 14-15 in female	19-23 (basically 21) in male, 20-21 (basically 21) in female	20-23 in male, 19-21 in female
No. of maxillary palpus segments	Palpus rudimentary	4 (segments small)	4	6	5
No. of labial palpus segments	Palpus rudimentary	2 (segments small)	2	3	3
Pronotum	Normal	Anteriorly overhanging	Anteriorly overhanging	Normal	Normal
Lower lateral lobe of pronotum	Normal	Normal	Normal	At least slightly incised	Distinctly incised
Shape of metanotum	Normal	Normal	Normal	Pyramidal	Normal
2nd & 3rd cubital cells	Not separated	Often not separated	Distinctly separated	Distinctly separated	Distinctly separated
Hind trochanter	2-segmented	2-segmented	2-segmented	2-segmented	2-segmented
Apical spur of foretibia	?	Absent	Simple	Apically bifid	Apically bifid
Female gastral shape	Rather flattened	Rather flattened	Rather flattened	Thick	Thick
Sternal armature (processes) in female	Present on 3rd	Present on 3rd	Present on 3rd	Present on 2nd and 3rd	Present on 2nd and 3rd
Punctation on gastral tergites	Tergites smooth	Fine and superficial; tergites weakly shiny	Fine and superficial; tergites weakly shiny	Very fine and sparse; tergites almost smooth and very shiny	Fine but distinct and dense; tergites mat and weakly shiny
Hosts	Agelaia, Polybia, or Stelopolybia	Ropalidia	Parapolybia	Vespa, Vespula, Dolichovespula	Vespa, Vespula, or Provespa

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References

Bischoff, H. von. 1933. Beiträge zur Kenntnis der Trigonaloiden. Mitteilungen aus dem Zoologischen Museum in Berlin 19: 480-496.

Bischoff, H. von. 1938. Trigonaloidae. Hymenopterorum Catalogus 5: 1-18.

Cameron, P. 1897. New species of Hymenoptera from Central America. Annals and Magazines of Natural History 6 (19): 261-276, 368-379.

Carmean, D. 1988. Biology of the Yellowjacket Parasitoid Bareogonalos canadensis (Harrington) (Hymenoptera: Trigonalidae). Unpublished Master thesis, Oregon State University, Corvallis, Oregon, USA.

Carmean, D. 1991. Biology of the Trigonalidae (Hymenoptera), with notes on the vespine parasitoid Bareogonalos canadensis. New Zealand Journal of Zoology 18: 209-214.

Carmean, 1995. Trigonalidae. In: Hanson, P.E. and Gauld, I.D. (eds.), The Hymenoptera of Costa Rica. Oxford Science Publications/ The Natural History Museum, Oxford and London: 187-191.

Carmean, D. and Kimsey, L. 1998. Phylogenetic revision of the parasitoid wasp family Trigonalidae (Hymenoptera). Systematic Entomology 23: 35-76.

Chen, H-Y., van Achterberg, C., He, J.-H. and Xu, Z.-F. 2014. A revision of the Chinese Trigonalidae (Hymenoptera, Trigonaloidea). ZooKeys 385: 1-207.

Clausen, C.P. 1931. Biological notes on the Trigonalidae (Hymenoptera). Proceedings of the Entomological Society of Washington 33: 72-81.

Clausen, C.P. 1940. Entomophagous Insects. New York: MacGraw-Hill. 342 pp.

Haneda, Y. 1993.An observation on the initial nest of Vespa analis insularis Dalla Torre (Hymenoptera: Vespidae). Fukui-chuho 13: 53-54. (In Japanese.)

Itami, H. 2011. Records of Trigonalidae in Niigata Prefecture. Tsunekibachi 19: 35-36. (In Japanese.)

Iwata, K. 971. Ethological notes on four species Japannese species of Vespa. In Entomological essays to commemorate the retirement of Prof. K. Yasumatsu (Asahina A., Gressitt J.L.,Hidaka., Nishida, T. and Namura, K., Eds), Hokuryukan, Tokyo: 219-223.

Iwata, Y. and Fukada, J. 2010. Records of Bareogonalos jezoensis (Uchida) (Hymenoptera, Trigonalidae) from Niigata Prefecture, Honshu, Japan and their percentages of parasitism in the nests of Vespa simillima Smith (Hymenoptera, Vespidae). Tsunekibachi 18: 27-30. (In Japanese.)

Lelej, A.S. 1995. Fam. Trigonalidae. In: Kupianskaya A.N., Lelej, A.S., Strozheva N.A. (eds.), Key to the Insects of Russian Far East. 4 (2): 8-14. (In Russian.)

Lelej, A.S. 2003. A review of the family Trigonalidae (Hymenoptera) of the Palaearctic Region. Far Eastern Entomologist 130: 1-7.

Makino, S. 1982. Nest structure, colony composition and productivity of Dolichovespula media media and D. saxonica nipponica in Japan (Hymenoptera, Vespidae). Kontyû 50: 212-224.

Matsuura, M. 1995. Social Wasps of Japan in Color. Hokkaido University Press, Sapporo. 353 pp. (In Japanese with English Summary.)

Matsuura, M., and Yamane, Sk. 1984. Biology of the vespine wasps. (1990 English Language edition), Springer-Verlag. Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong. 323 pp.

Nakamura, M. 1978. [Records of Bareogonalos jezoensis]. Hymenopterists' Communication, Fukui 7: 16-17. (In Japanese.)

Nozaka, C. 1976. [Observations on the oviposition behavior of Bareogonalos jezoensis]. Hymenopterists' Communication, Fukui 3: 1-4. (In Japanese.)

Ono, M. 1987. Note on Bareogonalos jezoensis (Hymenoptera, Trigonalidae) discovered from a nest of Vespa simillima xanthoptera (Hymenoptera, Vespidae). Kontyû 55: 162-163.

Ono, M. 1988. A new host record of Bareogonalos jezoensis (Hymenoptera, Trigonalidae) from Japan. Kontyû 56: 191-192.

Popov, V.V. 1945. Zoogeography and some morphological peculiarities of the family Trigonaloidae (Hymenoptera). Compt Rendus (Doklady) de l'Academie des Sciences de l'URSS NS 48 (1): 72-74.

Richards, O.W. 1977. Hymenoptera. Introduction and key to families. Handbooks for the Identification of British Insects. London: Royal Entomological Society of London. 6 (1): 100 pp.

Riek, E.F. 1970. Hymenoptera. In: The Insects of Australia. CSIRO, Camberra. 879-881pp.

Sayama, K. 1999. Notes on an embryo nest of Vespa analis Fabricius (Insecta: Hymenoptera; Vespidae, Vespinae) parasitized by Bareogonalos jezoensis (Uchida) (Hymenoptera; Trigonalidae). Natural History Bulletin of Ibaraki University 4: 1-2.

Schulz, W.A. 1907. Genera Insectorum. Hymenoptera, Fam. Trigonaloidae. (ed. by P. Wytsman) 61: 1-24.

Tsuneki, K. 1973. On Trigonaloidea of Japan. Life Study. Fukui 17: 128-130. (In Japanese.)

Tsuneki, K. 1991. Revision of the Trigonalidae of Japan and the adjacent territories. Special Publications of the Japan Hymenopterists Association 37: 1-63.

Uchida, T. 1929. Uber die Trigonaloiden Japans. Insecta Matsumurana, 3: 77-80 (Taff. II).

Vecht, J. van der. 1933. Notes on Nippogonalos jezoensis Uchida. Tijdschrift voor Entomologie 76: 78-82.

Weinstein, P. and Austin, D. 1991. The host relationships of trigonalyid wasps (Hymenoptera: Trigonalidae), with a review of their biology and catalogue to world species. Journal of Natural History 25: 399-433.

Weinstein, P. and Austin, A.D. 1996. Thelytoky in Taeniogonalos venatoria Riek (Hymenoptera:Trygonalyidae), with notes on its distribution and first description of males. Australian Entomology 35: 81-84.

Westwood, J.O. 1868. Descriptions of new genera and species of exotic Hymenoptera. Transactions of the Entomological Society of London 1868: 327-332.

Yamane, Sk. 1973. Descriptions of the second to final instar larvae of Bareogonalos jezoensis with some notes on its biology (Hymenoptera, Trigonalidae). Kontyû 41: 194-202.

Yamane, Sk. and Kojima, J. 1982. Pseudonomadina new genus, with a description of a new species from the Philippines (Hymenoptera, Trigonalidae). Kontyû 50: 183-188.

Yamane, Sk. and Terayama, M. 1983. Description of a new subspecies of Bakeronymus typicus Rohwer parasitic on the social wasp Parapolybia varia Fabricius in Taiwan (Hymenoptera: Trigonalidae). Memoirs of the Kagoshima University Research Center for the South Pacific 3: 169-173.

Yamane, Sk. and Yamane, So. 1975. A new trigonalid parasite (Hymenoptera, Trigonalidae) obtained from Vespula nests in Taiwan. Kontyû 43: 456-462.

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A practical guide to DNA extraction, PCR, and gene-based DNA sequencing in insects

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Abstract

Molecular methods have been highly informative for inferring evolutionary relationships, implementing diversification and biogeographic range evolution analyses, and inferring the evolution of traits of interest. Although these molecular laboratory methods are widely used across the tree of life, there are specific protocols that are useful within taxonomic groups. For these reasons I present protocols I have successfully used for DNA extraction, polymerase chain reaction (PCR), and cycle sequencing for gene-based and targeted genome sequencing of insects. This practical guide is not exhaustive and I encourage scientists to use, modify, and share these detailed steps as needed for their taxonomic group of interest or specific scientific question.

Keywords: DNA extraction, PCR, gene, sequencing, insects, protocol.

Introduction

Molecular methods are powerful tools for inferring the evolutionary relationships/systematics across the tree of life and implementing further diversification analyses. As many entomologists are interested in using molecular methods to infer evolutionary relationships of their groups of interest, here I present practical methods for genomic DNA extractions, the polymerase chain reaction (PCR) to amplify target genetic/genomic regions of interest, and cycle sequencing for Sanger-based DNA sequencing (Figure 1). This how-to guide is not meant to serve as an exhaustive explanation of all the possible methods or techniques, but only to outline the methods that I have been able to successfully implement (as in Saux et al. 2003; Saux et al. 2004; Moreau et al. 2006; Moreau 2008; Moreau 2009; Moreau 2011; Moreau & Bell 2011; Sarnat & Moreau 2011; Moreau & Bell 2013).

An additional concern of entomologists it how to preserve specimens in the field for future molecular analysis. For a review and quantitative analysis of commonly used preservatives for insects for molecular methods, please see Moreau et al. (2013) and the references within. In short, high percentage ethanol (95-100% EtOH) is ideal, although propylene glycol can be an effective preservative for passive traps or when there are safety concerns regarding the use and/or transport of ethanol.

Lastly, although these methods have been successful under the conditions, which I have used them there will always be exceptions and steps to improve these protocols for your specific group of interest or research question. I encourage you to modify (and share widely) the protocols outlined below. In addition, although many scientists are moving toward next-generation sequencing to sample either the reduced genome or whole genomes to address questions in systematics and evolutionary biology, for many scientists targeted genome and gene-based Sanger sequencing will likely remain a tractable, affordable, and useful method for many scientific questions and to infer the phylogenetic relationships of understudied taxonomic groups.

DNA EXTRACTIONS USING QIAGEN DNEASY KITS

(Online: 15-November-2014; Print: 9-January-2015)

These are the instructions I use for DNA extractions of individual or parts of ants, but are useful for almost any insect or tissue (invertebrates to vertebrates). They are rather comprehensive as they were written so that someone with any level of laboratory experience can follow the protocol.

These instructions are for extracting DNA from 24 or fewer ant specimens (number determined by size of microfuge centrifuge capacity; increase accordingly if necessary, but I suggest starting with much fewer samples to limit the chances for mistakes) using the Qiagen DNeasy DNA extraction kit and tungsten carbide beads and extraction machine, but this protocol can be easily modified to accommodate other kits or physical cuticle disruption.

Each individual collection or specimen must have a unique number so you can associate the voucher (which should be deposited in a curated university or museum collection) with each DNA extraction. Voucher specimens can take the form of an individual separate from the individual sacrificed in the DNA extraction (this works wel for social insects from the same nest series) or a specimen missing a specific body region (i.e. single leg or all the legs from one side of the body) if you only have a single specimen. First create a list of the specimens from which you will be extracting DNA (use an extraction worksheet or write in your lab notebook). You must be very diligent to never mix up a number or tube as this will lead to confusing the DNA of one specimen for another specimen. If you ever realize you have done this, it may be necessary to throw out the entire DNA extraction. Be sure to note this on your extraction worksheet or lab notebook.

Before you begin the DNA extractions it is very important to make sure everything you will be using is DNA free.

- Get six 96 tube racks, forceps, small beaker, Petri dish, and anything else you use for the protocol and put under the UV light bench for 15 minutes or longer (the UV will glow bluish when turned on) or soak in a 10% bleach solution and then allow to dry overnight. The bleach and/or UV light destroys DNA that could contaminate your new extractions.
- Once you are ready to begin, thoroughly clean your lab bench with 10% bleach and ethanol. Be sure it is entirely dry before putting your extraction materials on the bench.
- When above materials are clean and DNA-free, bring them to the lab bench with new, gloved hands.
- Check that the hybridization "rotisserie" oven or oven with "centrifuge tube rocker" is turn on and set at 55-56˚C.

Next make sure you are set up to begin the DNA extractions.

- I find that if you stagger the tubes in the racks by putting eight tubes in the first row of the rack in positions 1, 3, 5, 7, 9, 11, 13, 15 and then skip a row and then add the next eight tubes in positions 2, 4, 6, etc. and then the last eight tubes in the last row in positions 1, 3, 5, etc. this will allow room for opening all the tubes without the lids getting too much in the way.
- As you open the 1.5mL tubes (now and in all future steps) be careful not to put your thumb or fingers on the inside of the lid (you can introduce contamination this way).
- Put the vials currently holding the specimens you intend to extract in one of the newly sterilized 96 tube racks in the order in which you have entered them on your extraction sheet.
- In the next sterilized rack put one clean, lid-closed 1.5mL tube for every specimen you will be extracting in the 96 tube rack. Number these tubes on the top using a lab pen (Sharpie, VW pen, etc.) with the same numbering system as the worksheet (does not have to be the collection code; can be a number 1-24, etc.)
- Next put the same number of Qiagen extraction filter tubes in the next 96 tube rack (you will have to peel each tube

from its individually sealed holder). Number these with the same system you numbered the 1.5mL tubes above (i.e. 1-24, etc.)
- In the next two 96 tube racks set up the same number of empty Qiagen collection chambers/tubes as the number of extractions you are doing.
- In the last rack place closed 1.5mL tubes (again the same number as you are extracting). It is ideal to label these with the "Tough-Spots" stickers, as this will prevent the number from being accidentally rubbed away, writing the collection code from your extraction sheet (not #1-24, etc., but the actual unique specimen collection codes). These numbers should be the same collection codes as found on the vials/tubes you are taking the whole specimens from and correspond to the collection locality data for specimen you are extracting.

You are now ready to begin the DNA extractions.

- Be sure to have a box of sterile lab wipes ready (i.e. KimWipes, etc.).
- Make sure all your tubes are always in the same order as your extraction worksheet/lab notebook. This will help you to minimize errors.
- Open each of the 1.5mL tubes you labeled #1-24 with the lids opened away from you again being careful not to let your thumb or fingers touch the inside of the lid (these are the vials you will be placing the specimen into and adding the Qiagen chemicals to leave from three hours to overnight).
- Fill your Petri dish with sterile PCR water.
- Fill your small beaker with 95% ethanol and place forceps in this beaker (you will use these to take the individual insect specimens from the tubes).
- Begin with the first vial of specimens. Take one single insect specimen out of the vial using the forceps and place in the Petri dish of water, insuring the size and depth of your dish is appropriate to fully submerge your specimen.
- Leave in the water for 10 seconds to remove any ethanol on the specimen from the collection/storage tube. No need to shake around.
- Remove the insect specimen from the PCR water using the forceps and place gently on a clean lab wipe.
- Leave on lab wipe for a few seconds to get most to all of the water off.
- Place the insect specimen in a labeled, open 1.5mL tube corresponding to the correct extraction number. Always check everything twice!
- Once you have placed an insect specimen in the open 1.5mL tube, leave the tube open to allow to the insect specimen to dry, but turn cap towards you to indicate that this vial now has an insect specimen inside (this will help you to not accidentally put two insects in the same vial).
- Clean forceps in the beaker of ethanol and wipe with a clean lab wipe.
- You are now ready to begin with the next specimen.
- Continue until all insect specimens are in an individual 1.5mL tube.

With one single insect specimen in each tube, you are now ready to move to the next step.

- If using the Qiagen tungsten carbide bead and Qiagen extraction machine use the following steps, otherwise physically homogenize the specimen with a sterile Teflon pestle in the first extraction kit solution (both methods work well):
- Again clean your forceps in the beaker of ethanol and wipe clean with a lab wipe.
- Get a vial of clean Qiagen tungsten carbide beads (or a clean pestle if you do not have Qiagen tungsten carbide beads).
- Place one bead in each tube with the insect specimen and close the tubes.

- Load the vials with the insect specimen and tungsten carbide bead into the Qiagen extraction machine (be sure to load both racks equally – they must be balanced) (or grind each specimen with a clean pestle).
- You will turn on machine for 20 seconds (no more than 20 seconds) at 30.0 1/s frequency.
- Take the tubes with the now shattered ant with the metal bead inside and place back in the rack in numerical order (again staggering the tubes in the rack).

You will now begin to follow the Qiagen DNA extraction instructions (be sure to always use barrier/filtered tips for pipetting).

- Open all the tubes (again with the lids open away from you).
- As you are opening each tube, check the lid to see if the metal bead cracked the lid (this happens rather frequently). If a tube has a cracked lid, carefully cut off the lid at the plastic hinge making sure not to lose the material inside after you have added the buffer and ethanol (outlined below) and vortexed, etc. Then cut the lid off a new tube and place on this tube. Be sure to label with the same exact number that was on the tube before you cut the lid off.
- Add 180uL of Buffer ATL to each tube.
- Add 20uL Proteinase K (ProK; >600 mAU/ml) to each tube (pipetting the mixture up and down), closing each tube as you finish this step.
- Once you have added the Buffer ATL and ProK to each tube, place each tube in the hybridization oven at 55-56˚C.
- Turn "rotisserie" or "rocker" on so the tubes are rotating or rocking while they are "cooking" at 55-56˚C. If you do not have an oven that can slowly rotate or rock your samples, you can physically shake the samples every half hour or so to insure mixing during this step.
- Leave tubes in the hybridization oven for three hours minimum to overnight (but not more than 30 hours as your samples may begin to evaporate).

After three hours to overnight, you are ready to finish the DNA extractions.

- Remove the tubes from the "rotisserie" and place back in the 96 tube rack.
- Get a small Erlenmeyer flask from the glassware cabinet (you will place the "dirty" metal extraction beads in here).
- Now follow the instructions in the Qiagen kit.
- Add the Buffer AL to each tube as per the instructions and vortex. Then add the 100% ethanol as instructed and vortex. After you pipette off this mixture (using a barrier/filtered tip) and add to the Qiagen filter containing extraction tubes, dump the "dirty" tungsten carbide bead in a small Erlenmeyer flask (you will clean these when you are finished the DNA extractions).

Notes about the Qiagen extraction kit:

- Always spin your tubes in the centrifuge with the hinges angled down. You must always put your hinges in the same orientation in the centrifuge to ensure maximum DNA recovery.
- On the last step, only add 200uL Buffer AE once. Do not do this step a second time as suggested in the instructions.
- Also once you have added the 200uL Buffer AE, wait 10 minutes before spinning in the centrifuge for the final step. This insures that maximum DNA is removed from the column.
- When spinning in centrifuge for final spin with collection barrier tubes placed within labeled final tubes, only put a tube in every other centrifuge hole and make sure the caps of the final tubes are not overlaid or they will snap off. If this occurs make sure you know which caps belong to which samples before taking any of the tubes out of the centrifuge.

Cleaning the Qiagen tungsten carbide beads.

- UV or bleach the forceps and a container for 15 or more minutes to hold

the metal extraction beads in the last steps (the plastic lid of a 96 well plate will work or the lid of an empty tip box).

- After placing all the "dirty" metal extraction beads in the small Erlenmeyer flask, add some distilled water and swish around. Get an empty beaker to pour into. As you will want to pour off the water and not lose any of the metal beads, pour this into the beaker so that any that fall out land in the beaker and not down the sink.
- Once you have poured off as much water as possible without pouring the metal beads out, get three lab wipes and fold in half and place over mouth of Erlenmeyer flask. Then dump over so the lab wipes to absorb the excess water (repeat if necessary, but beads do not have to be completely dry).
- Next add 0.4M HCL to the Erlenmeyer flask and "dirty" metal beads filling about ¼ full.
- Agitate in the solution for about 1-2 minutes to clean metal extraction beads (some may appear tarnished, but this is fine).
- You must pour this 0.4M HCL into a properly labeled waste jar under a fume hood. Again be careful to not lose the beads (you can leave a little of the HCL in the Erlenmeyer flask if you are worried you will lose the beads).
- Now add a little distilled water and again swirl around to wash the beads. Carefully pour off water making sure to not lose the metal beads in sink. Again use three folded lab wipes over the mouth of the Erlenmeyer flask to absorb last of the water.
- Now line the container (a sterilized empty tip container will work fine) with several lab wipes. Dump the beads in and rub them with another clean lab wipe.
- Now put container and metal extraction beads under the UV light for 15 minutes if available, otherwise soak them in 10% bleach solution for 5 minutes (or longer) insuring they are completely dry before proceeding to the next storage step. Every 5 minutes or so roll the beads around to expose all sides to the light.
- Once done, using forceps place beads in a new, clean 1.5mL tube. Only put 24 metal beads in each tube so they are ready for your next round of DNA extractions.

Special notes:

- If you ever are worried that you have touched something that can possibly contaminate your samples, always throw away your gloves and get new ones.
- Always use barrier/filter tips for DNA extractions (remember always use barrier/filter tips for any lab work up to and including PCR).
- It is best to do all DNA extractions in clear (non-colored) tubes. This allows for the visualization of contaminants in your tube such as pigments from the insect specimens or other items.
- If you are worried you may have ruined or contaminated an individual tube or sample, make a note of it on the worksheet. This will help narrow down potential problems later.
- Many of the Qiagen buffers have very similar initials/names, so be extra careful you are using the correct buffer in the correct order.
- Remember there is no such thing as being too careful in the lab.

Storage of your DNA extractions:

If you are using your DNA extractions often, it is best to store them in a standard refrigerator (~4˚C). For long-term storage it is best to place your DNA extractions in a non-frost free freezer (as these maintain the lack of frost by slightly warming and cooling the freezer) or deep freezer. Remember that repeated freezing and thawing your DNA samples can result in shearing of the DNA strands.

DNA EXTRACTION USING PHENOL/CHLOROFORM

The grinding solution is made from the following stock solutions:

	1 Sample	10 Samples
Water (PCR Grade)	305µL	3.05mL
0.5 M EDTA	100µL	1.0mL
0.1 M Tris (pH 8.0)	50µL	500µL
20% SDS	25µL	250µL
Proteinase K (20mg/ml)	20µL	200µL
Total	500µL	5.0mL

- Homogenize sample with Teflon pestle in grinding solution.
- Incubate the homogenate at 55°C for 3 hours (or overnight).
- Add 550µL of equilibrated phenol to the sample.
- Vortex for 1 minute. Microfuge for 5-10 minutes.
- Take off the supernatant (which contains the DNA) and place in new tube.
- Discard old tube or save to ensure DNA was not lost.
- Repeat steps 1-3 about 2-3 times, or until the supernatant is not cloudy and discolored.
- Add 550µL of chloroform to each sample.
- Vortex for 1 minute. Microfuge for 5-10 minutes.
- Take off the supernatant (which contains the DNA) and place in a new labeled tube.
- Discard old tube. Repeat steps 5-7 again (optional).
- Add 750µL of cold 100% EtOH to the supernatant and let stand for 2 hours at -20°C (can be overnight).
- Microfuge for 10 minutes to pellet the DNA and gently discard the supernatant by slowly pouring it off, leaving only the pellet.
- Gently add 50µL of cold 70% EtOH to the pelleted DNA.
- Microfuge for 10 minutes and gently discard the supernatant by slowly pouring it off, leaving only the pelleted DNA. Repeat steps 10 and 11 again.
- Dry pelleted DNA in speedvac or invert and allow to dry completely.
- Resuspend the dry pelleted DNA in 200µL 0.1 mM Tris pH 8.0 or 200µL PCR water.

PCR PRIMER SELECTION

Selection of genes/genomic regions for amplification and DNA sequencing and the PCR and cycle sequencing primers that are most appropriate will depend not only on your taxonomic group of interest, but also on the evolutionary age or depth of the group. I encourage you to read through studies related to your taxonomic group of interest and potentially investigate the genomic structure of any available genomes available to determine the appropriate level of molecular variation for your taxonomic group and the amount of molecular variation necessary to answer your scientific questions.

POLYMERASE CHAIN REACTION (PCR)

Most of the protocols to set up your PCR reaction are highly similar to those outlined below in the cycle sequence reaction section. You will need to vary the size of your reactions depending on the number of the samples you have extracted for amplification. I will not outline in detail how to set up a PCR reaction here since most students have the opportunity to do this during their studies. I will present the specific parameters I have used for insects, but if you need additional detail you can coopt the details below in the cycle sequence reaction section. Keep in mind if you add other chemicals to improve the efficiency of your chemical reaction you will need to reduce the amount of water in the reaction to keep the reaction at the same volume. Also note that to accommodate pipette error, it is advisable to set up your master mix for one or two more reactions that you are actually doing.

PCR master mix protocol (25 µL per sample master mix solution):

Water (pure, DNA free water)	to 25 µL
10x buffer	2.5 µL
$MgCL_2$ (25 mM)	1.5 µL
dNTPs (8 mM)	2.5 µL
Forward Primer (10µM)	1.2 µL
Reverse Primer (10µM)	1.2 µL
DNA Template	1 µL
Taq polymerase(5U/µL)	0.1-0.2 µL (or less)

PCR thermal cycler parameters:

Step 1: Initial Denature	94˚C for 1 minute
Step 2: Denature	94˚C for 30 seconds
Step 3: Anneal	varies, but usual 48-58˚C for 1 minute
Step 4: Extension	72˚C for 2 minutes
Repeat steps 2-4:	30 times
Step 5: Final Extension	72˚C for 3 minutes

Storage of your PCR products:

Although not particularly temperature sensitive once your PCR is complete, it is best to store your PCR product in a standard refrigerator (~4˚C) to prevent evaporation.

PCR TROUBLESHOOTING

If your PCR sample does not amplify (or amplifies very weakly) try these steps in order:

- If your sample does not work at all the first time, try again with the exact same parameters and protocol.
- If your sample still does not work at all or amplified very weakly, next lower your annealing temperature by 2-5˚C (i.e. If you originally amplified at 54˚C try re-amplifying at 52˚C), but keep all else the same.
- If lowering by 2˚C does not work, try lowering the annealing temperature again by another 2-3˚C.
- If lowering the temperature does not work, try adding more $MgCl_2$. Add 1µL more $MgCl_2$ per reaction.
- In some rare cases with nuclear genes, raising (not lowering) the annealing temperature actually helps. Try raising the temperature by 2-5˚C from the original annealing temperature.
- You may also consider including an additive in your PCR (or changing the additive if you are currently already using one). Examples include BSA, DMSO, etc.

If a sample is not amplifying for multiple genes using the steps above:

- First try adding additional DNA template to your PCR reaction (i.e. if you originally were using 1µL per reaction, try adding 2-4µL per reaction).
- If you have reason to believe there is too much DNA in that particular extraction (i.e. this was the largest of your ants, beetles, muscle tissue, etc.), dilute a subsample of your DNA extraction by half with PCR water (i.e. 20µL original DNA extraction and 20µL PCR water) and use this as the template for the PCR.
- If the steps above do not work, re-extract that sample if you have additional material available.

If your PCR sample amplifies but sequences poorly in both directions:

- Re-PCR with a higher annealing temperature. If the samples still amplifies well (bright band on gel) try sequencing this product.
- If after amplifying with a higher annealing temperature this does not improve your sequence quality but your samples still amplify, then try raising the temperature again.
- If you still have low quality sequences, try reamplifying with less $MgCl_2$.

Special notes:

- You should always use barrier/filter tips for all PCRs if possible to reduce the amount of possible cross contamination.
- To get strong clean DNA sequences you must start with good PCR product.
- Remember lowering the annealing temperature and adding $MgCl_2$ both decrease the specificity of your reactions, so this can lead to amplifying

non-target regions of the genome or contaminates.
- Never amplify your PCRs below an annealing temperature of 45°C.
- Again remember there is no such thing as being too careful in the lab.

GEL ELECTROPHORESIS AND PCR CLEANING

Before proceeding to the cycle sequencing step, it is highly advisable to use gel electrophoresis to visualize your PCR product. This insures that you have 1) successfully amplified a product; 2) amplified the target PCR product size; and 3) permits confirmation of a single amplified PCR product (as long as the non-target product size is significantly different in length).

In addition, you must clean your PCR product to remove residual primers and unincorporated nucleotides. There are many commercial products available to complete this step.

CYCLE SEQUENCING, CLEANING, AND LOADING SAMPLES IN A SINGLE 96-WELL PLATE

To save time, money and lab supplies, you can cycle sequence your samples in the plate you are will actually load into the sequencing machine.

Cycle sequencing master mix protocol for each direction/single primer (10 µL per sample master mix solution):

5x buffer	1.65 µL
BigDye Terminator (BDT)	0.75 µL (or less)
Primer (10µM)	0.3 µL
DNA Template	1-4 µL
Water	to 10 µL

Cycle sequencing thermal cycler parameters:

Step 1:Initial Denature	96°C for 1 minute
Step 2: Denature	96°C for 10 seconds
Step 3: Anneal	50°C for 5 seconds
Step 4: Extension	60°C for 4 minute
Repeat steps 2-4:	25 times

To set up your cycle sequence reaction:
- Start with a clean 96-well plate (this can be a plate that has been used previously, but should be completely clean and dry before you begin.)
- You will need a silicon 96-well mat to cover your samples when you are finished, which should also be clean and dry (these mats can be used over and over).
- Make sure you have all your cleaned PCR samples ready (don't forget to quickly spin these down if they have been in the refrigerator, as there will likely be condensation on the lids).
- Put your 96-well plate on ice (only to prevent evaporation of your DNA template – the cleaned PCR product - once you load them into the wells of your 96-well plate).
- Make up your cycle sequencing master mix minus the Big Dye Terminator (BDT), which you should leave in the freezer until you are ready to use it since BDT is light sensitive. You will add the BDT at the very end to the master mix, but after you have your template in the wells.
- Put your master mix minus the BDT on ice.
- Add your DNA template (the cleaned PCR product) to each well of your 96-well plate. Be sure to load the plate from 1A – 1H then to 2A – 2H, 3A – 3H, etc. ending on well 12H.
- Tip – I usually add the template to the front inside of the tube/well, touching the pipette tip to the inside front side of the tube to insure the droplet of template does not stick to the outside of the pipette tip.
- Once you have your DNA template in each of the wells, get your BDT out of the freezer and add it to your master mix. Be sure to mix well by pipetting up and down several times.
- If you have added your template to the front inside of the wells, then you can

add your master mix to the back inside of each well, allowing the pipette tip to touch the inside of the well without chance of contamination.

- After you have added the master mix to each well, cover with the clean, dry silicon mat and quickly spin down your plate in the plate centrifuge to insure that your DNA and master mix are both in the bottom of the wells (be sure you have another mat covered plate to use as a balance).
- After placing in the thermal cycler, be sure to put out a box with aluminum foil to cover your plate, as BDT is light sensitive.

Storage of your cycle sequence reactions:

Although not particularly temperature sensitive cycle sequencing is light sensitive. For this reason it is best to wrap your samples in aluminum foil to protect them from light. Once your cycle sequencing is complete, you can leave them in the refrigerator for up to a week before cleaning and Sanger sequencing, as long as it remains in the dark.

Cleaning your cycle sequence reaction (EtOH/EDTA precipitation):

- Before you begin, quickly spin down your plate (making sure to balance the centrifuge) as there is likely condensation on the lid/mat. Note orientation of mat on 96-well plate so you can put it back on in the same orientation.
- Make a master mix of the EtOH/EDTA solution in a plastic trough so you can use a multi-channel pipette to dispense this solution into your 96-well plate. For a 10µL reaction, you will need to add 30µL 100% EtOH and 2.5µL 125mM EDTA, so for a 96-well plate make up the following:

  3300µL 100% EtOH
  275µL 125mM EDTA
- Mix solution by pipetting the solution up and down a few times.
- Add 32.5µL of the EtOH/EDTA solution to each well of your 96-well plate using a multi-channel pipette (you will only need to use one set of pipette tips if you do not touch the tips to the 96-well plate).
- Seal 96-well plate with silicon mat (again paying special attention to place the mat back on the tray in the same orientation as you took it off) and quickly vortex to mix.
- Leave at room temperature for 10-15 minutes in a dark location (i.e. in a drawer) or covered in aluminum foil.
- Spin in refrigerated (if available) centrifuge at 2500g for 30 minutes at 4°C. Be sure to balance centrifuge.
- Important: Proceed to next step immediately (you must be ready and present as soon as the centrifuge stops). If not possible, you must spin the sample again for 10 minutes to re-pellet the product.
- Remove silicon mat (again noting orientation of mat on 96-well plate) and invert tray onto folded paper towel and place in centrifuge rack.
- Place tray inverted into centrifuge and spin 50g for 2-3 minutes.
- Add 30µL 70% EtOH to each pellet (just eyeball pouring 70% EtOH into trough to use multi-channel pipette to distribute). No need to mix or vortex.
- Seal 96-well plate with silicon mat (again in same orientation as original cycle sequence reaction).
- Centrifuge plate 2000-3000g for 15 minutes at 4°C.
- Important: Proceed to next step immediately (you must be ready and present as soon as the centrifuge stops). If not possible, you must spin the sample again for 10 minutes to re-pellet your product.
- Again, remove silicon mat and invert tray onto folded paper towel and place in centrifuge rack.

- Place tray inverted into centrifuge and spin 50g for 2-3 minutes.
- Place 96-well plate in 65°C oven for 10 minutes to allow to thoroughly dry.
- While waiting, clean gray septa mat cover and make sure it is completely dry.
- If you cannot load samples directly onto sequencing machine at this time, you can cover the 96-well tray with a clean silicon mat, wrap in foil and freeze until you are ready to sequence.

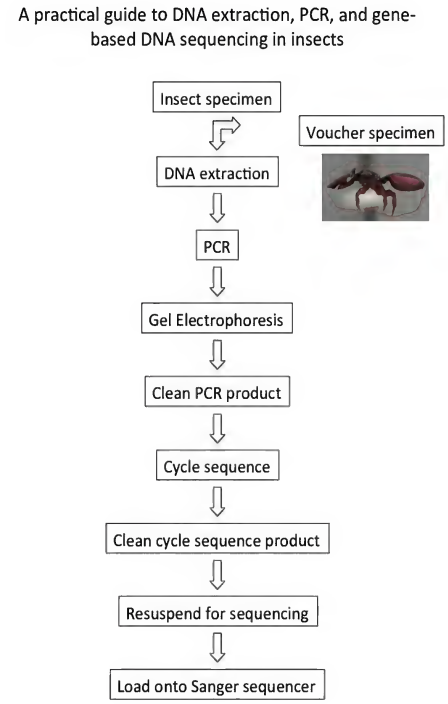
Resuspending samples in HiDi for sequencing

- Add 10µL to each well of the 96-well plate (if you have "blank" lanes without any DNA product, you must still load with HiDi or ddH_2O – there must be HiDi or ddH_2O in every well so as to not damage the capillary arrays of the sequencing machine).
- If your plate will be the first reaction on the sequencing machine, you must wait 10 minutes before placing your plate on the machine to allow for the pellet to resuspend in the water (if there are other plates ahead of your plate, you can place it directly into the queue).

Additional cycle sequencing notes:

- You do not need to use barrier/filter tips for cycle sequencing, except when you take aliquots from your stock reagents (like your primers) that are still used in your PCRs.
- For most cycle sequence reactions, there is no need to use more than 0.75µL of BDT per 10µL reaction (in some cases you may even be able reduce the amount of BDT to 0.50µL or 0.25µL per reaction). This will save you a substantial amount of money in the long run.
- If you only need to sequence a half plate, you must load your 96-well plate every other column (i.e. 1A – 1H, 3A – 3H, 5A – 5H, etc. ending on 11H), to insure the capillaries on the Sanger sequencer are immerged in the wells that contain your product.
- You can reuse your 96 well plates. Be sure to clean them thoroughly after they come off the sequencing machine, this includes washing with water and sterilizing when you have this option available. I have reused the same 96-well plates for more than year without any adverse effects.

Figure 1.Overview of the major steps of DNA extraction, PCR, and gene-based DNA sequencing in insects. Photograph of voucher specimen of Cephalotes varians by Gracen Brilmyer.



Conclusions

Molecular and genomic data provide a powerful and independent data source to address questions regarding the evolution of morphological characters, biogeography, diversification, and evolutionary relationships. My hopes with this detailed guide is to arm

scientists the world over to feel comfortable with the laboratory research protocols required to generate these data. In closing I hope this practical guide provides the foundation for future entomologists to leverage the power of DNA, genetics, and genomics to address questions in systematics and evolution.

Acknowledgements

Special thanks to Himender Bharti for inviting me to make these methods available in a single paper for the next generation of entomologists. Thank you to three anonymous reviewers for providing comments to improve this paper. In addition, thank you to the Negaunee Foundation for support.

References

Moreau, C.S., Bell, C.D., Vila, R., Archibald, S. B., and Pierce, N. P. 2006. Phylogeny of the ants: Diversification in the age of angiosperms. Science 312(5770): 101–104.

Moreau, C.S. 2008. Unraveling the evolutionary history of the "hyperdiverse" ant genus Pheidole (Hymenoptera: Formicidae). Molecular Phylogenetics and Evolution 48: 224-239.

Moreau, C. S. 2009. Inferring ant evolution in the age of molecular data (Hymenoptera: Formicidae). Myrmecological News 12: 201-210.

Moreau, C.S. and Bell, C.D. 2011. Fossil cross-validation of the dated ant phylogeny (Hymenoptera: Formicidae). Entomologica Americana 117(3): 127-133.

Sarnet, E.M. and Moreau, C.S. 2011. Biogeography and morphological evolution in a Pacific island ant radiation. Molecular Ecology 20: 114-130.

Moreau, C.S. 2011. What do molecular clocks tell us about the evolution of ants? (Invited Instant Symposium review) American Entomologist 57(1): 52-53.

Moreau, C.S., Wray, B.D., Czekanski-Moir, J.E., and Rubin, B.E.R. 2013. DNA preservation: A test of commonly used preservatives for insects. Invertebrate Systematics 27: 81-86.

Moreau, C.S. and Bell, C.D. 2013. Testing the museum versus cradle biological diversity hypothesis: Phylogeny, diversification, and ancestral biogeographic range evolution of the ants. Evolution 67(8): 2240-2257.

Saux, C., Fisher, B.L. and Spicer, G.S. 2004. Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae). Molecular Phylogenetics and Evolution 33: 457–468.

Saux, C., Simon, C.M. and Spicer, G.S. 2003. Phylogeny of the dragonfly and damselfly order Odonata as inferred by mitochondrial 12S rRNA sequences. Annals of the Entomological Society of America 96: 693–699.

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First record of the species Helochares atropiceus Regimbart, 1903 (Coleoptera: Hydrophilidae) from the Loktak lake of Manipur, India

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Abstract

Helochares atropiceus Regimbart, 1903 belonging to the genus Helochares Mulsant, 1844 (Coleoptera: Hydrophilidae) is recorded for the first time in India. The species Helochares atropiceus Regimbart, 1903 has been collected from Loktak Lake of Manipur and an identification key to the species of the Helochares species found in India is provided in the text.

Keywords: Hydrophilidae, Helochares, New records, Loktak Lake, Manipur, India.

Introduction

Hydrophilidae, a large family of beetles, is represented by 146 genera and about 3335 known species from the world (Hansen, 1999; Short and Herbauer, 2006; Short and Fikacek, 2011). From the four subfamilies of Hydrophilidae, only two (Hydrophilinae and Sphaeridiinae) are recorded from the Oriental region (Komarek, 2003), of which Hydrophilinae comprising more than 1600 described species is mostly aquatic. A total of 34 genera of aquatic Hydrophilidae are known from Oriental region. So far 23 genera and 67 species of Hydrophilidae have been recorded from India (Deepa, 2010).

Hydrophilid beetles are usually separated from other beetles as its first abdominal sternite is not divided by hind coxae, antennae terminating in an abrupt multisegmented club; maxillary palpi usually as long as and much longer than the antennae (except the subfamily Sphaeridiinae), pronotum mostly smooth and usually as wide as elytra at base; eyes usually not protuding but if so then the head usually deflexed. The species of this subfamily are generally found in stagnant waters and may also inhabit leaf litter and decaying organic material. Larvae are predaceous, preying on various smaller invertebrates, while adults are mostly saprophagous feeding on different kinds of decaying organic matters.

Helochares Mulsant, 1844 is a large genus of the Hydrophilidae. At present, the genus Helochares Mulsant, 1844 comprises 180 described species distributed worldwide (Hansen, 1999; Short and Herbauer, 2006; Short and Fikacek, 2011).

A total of 6 species of the genus have been recorded from India so far. Helochares can be separated from other Hydrophilids by the following characters; head not strongly deflexed; scutellum shorter than its basal width; meso and metatibia lacks swimming hairs. Antennae with nine segments, maxillary palp elongate longer than antennae, last segment shorter than the penultimates and pseudobasal segment of maxillary palps bowed inward when extended forward. Mesosternum without definite carina and all tarsi 5 segmented.

Materials and Methods

The material examined for this study was collected by means of sieve, ladle, and net with 1 mm pores in different sites of Loktak Lake of Manipur. The beetles were killed using ethyl alcohol solution. The specimens were studied in the Entomology Research Laboratory, P.G. Department of Zoology, Dhanamanjuri College of Science, Imphal. Aedeagophores were dissected under a stereo-zoom microscope and cleared in 10% KOH solution for 1-2 h. The photographs were taken using an Olympus type

(Online: 15-November-2014; Print: 9-January-2015)

BX51 compound microscope and a Nikon type SMZ 1500 stereo-zoom microscope. Three specimens were deposited in the Laboratory of Entomology, P.G. Department of Zoology, Dhanamanjuri College of Science, Imphal, Manipur. Two specimens were deposited in the Division of Entomology Museum. Indian Agriculture Research Institute (IARI), New Delhi, India.

Description

Helochares atropiceus Regimbart, 1903

Helochares atropiceus Regimbart, 1903, Ann. Soc. Ent. F. 72:53(Valid. sp., not syn. as in d'Orchymont, 1923, Treubia, 419.)

Helochares ohkurai Sato, 1976, Ent. Rev. Jap., 29:21. - Syn.; Hebauer, 2001, Latissimus, 14:15.

Material examined

2♂, Phubala (Loktak Lake, Manipur) wetland, 39 Km, South of Imphal, (Latitude 24° 27. 327´N and 93°51. 295´E Longitude), altitude763 m 21.xi.13. Coll. by M Bhubaneshwari, O.Sandhyarani and S.Dineshwar.

1♂&1♀ Longum (Loktak Lake, Manipur), wetland, 45 Kms, South of Imphal, (Latitude 24°31. 011´N and 93°49. 066´E Longitude), altitude 822 m 7.ii.2014. Coll. by, M.Bhubaneshwari, O.Sandhyarani and S. Dineshwar.

1♀ Takmupat (Loktak Lake, Manipur), wetland, 48 kms, South of Imphal (Latitude 24°29.221´N 93°48.571´E Longitude), altitude 800 m 13.i.2014. Coll. by, M. Bhubaneshwari, O. Sandhyarani, S. Dineshwar.

Differential diagnosis

Dorsal coloration generally black, medium size and generally fine punctuations on head, pronotum and elytra. Helochares atropiceus can be easily distinguished by its distinct shape of the aedeagus, small notch in front of the head and metasternal keel highest apically.

Form and colour

Body elongate oval, (Fig.1), length 6.48-6.50 mm and 2.99- 3.00 mm width, rather flat entirely black, shining, finely dense punctate on surface. Head, pronotum, scutellum and extreme base of the elytra black, lateral margins of pronotum, elytra narrowly pale, elytra black, maxillary palpi reddish brown, vertical surface of head brown, antennae reddish brown, thoracic and abdominal ventrites black, legs same colour as sternum and thoracic, generally punctuation on head, and pronotum moderately fine separated by about width of a puncture and punctuation on head and pronotum is similar to elytra and well impressed.

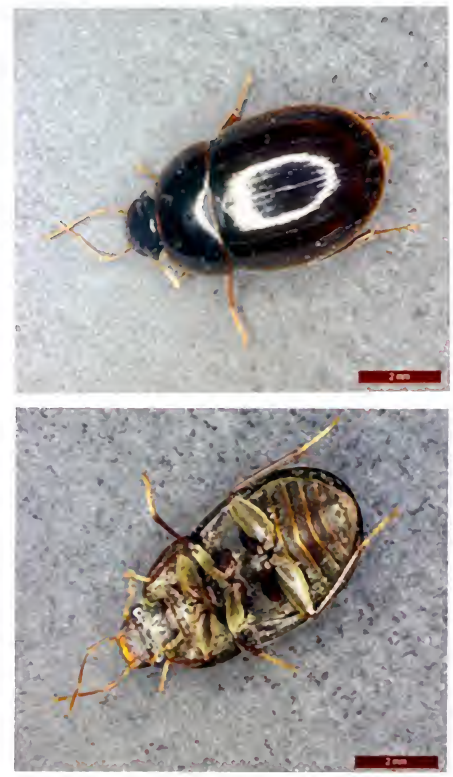

FIGURE 1.Habitus of Helochares atropi ceus (Dorsal & Ventral view)

Head

Front edge of the head sinuate, moderately fine and densely punctate, clypeus deeply excavated at anterior margin, maxillary palpi long and slender, second segment largest, apical segment about 2/3 length of the middle one, antennae 9 segmented with club fuscescent, the terminal joint twice as long as the preceding one.

Pronotum

Pronotum shining black, sides widely rounded margins narrowly dark reddish and a little less densely punctuate than head. Small to medium

size puncture, well impressed and separated by about width of a puncture.

Elytra

Elytra punctate same size or somewhat smaller than those of pronotum and a little sparser, particularly towards sides and apex. A sparse row of serial punctures traceable in about middle of each elytron and another weaker row near lateral margins, metasternum with a small elevation on middle. Femora with rugose portion covering all about small portion near apex, coxal plates sparsely and weakly punctured, covered in moderately impressed fine reticulation. Claws on protarsi strongly recurved, inner one with large scale like vertical expression at its base, metatarsal claw similar in shape but not quite as developed.

Abdomen

Abdomen with five exposed sternites, sternites shiny, covered with relatively sparse, small setose, punctures and apex of last sternites having with small notch.

FIGURE 2: Male genitalia of Helochares atropiceus (Dorsal & Ventral view)

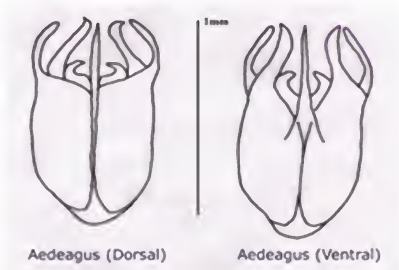

FIGURE 3: Male Aedeagus of Helochares atropiceus

Male genitalia

Aedeagus short and stout, from base wing shaped bent outwards terminally narrowed tips near parameres, parameres converging towards median lobe and two small hook shaped lobes present near median lobe. Median lobe narrow spine like as long as parameres (Fig. 2 & 3).

Biology

All the specimens were collected from the edge of lake Loktak which is unkempt and surrounded by marsh grasses and plant debris.

Remarks: This species is recorded for the first time from Manipur (Loktak lake) and also from India.

Discussion

Helochares atropiceus could be distinguished from other Helochares species on basis of short and stout aedeagus, base wing shaped, bent outwards, terminally narrowed tips near parameres, parameres converging towards median lobe and two small hook shaped lobes present near median lobe. Median lobe narrow spine like as long as parameres. Helochares atropiceus was distinguished from other Helochares based on male aedeagus structure provided by Jia Feng-long et al. (2010). d´Orchymont (1923) synonymised Helochares atropiceus and Helochares taprobanicus. But in present study Helochares atropiceus is not synonymised with Helochares taprobanicus being different in their male aedeagus, black maxillary palp, margins of pronotum fully black in colour. So, Helochares atropiceus and Helochares ohkurai Sato (1976) are synonyms according to Hebauer (2001).

Helochares species found in India:

1. Helochares pallens
2. Helochares taprobanicus
3. Helochares anchoralis
4. Helochares crenatus
5. Helochares densus
6. Helochares lentus
7. Helochares atropiceus (New record)

Tentative identification Key to the Indian Helochares species

The following key summarized the diagnostic characters of the Helochares species recorded from India. However, there might be at least as many undescribed species of the genus occurring in different part of India based on our inspection of several collections. The following key is therefore very tentative and should be used with extreme care.

1. Body longer than 4.0mm, dorsum with course ground punctuation or serial punctures; Coloration variable……..……2
- Body less than 4.0 mm long, dorsum with fine ground punctuation, without serial punctures; light brown in colour……..……...**H. pallens Macleay**
2. Elytra with distinct rows of coarse serial punctures, dorsal colouration light to medium brown……..……...……..4
- Elytra without distinct rows of serial punctures, dorsal coloration black……...……....3
3. Maxillary palpi black in colour and terminal joint much shorter than the third joint ……**H. taprobanicus Sharp**
- Maxillary palpi not black in colour and terminal joint not much shorter than the third joint…….**H .atropiceus Regimbart**
4. Elytra with moderately distinct rather fine punctuation and also with very distinct series of much larger punctures……**H. lentus Sharp**
- Elytra with less coarse and less punctuation……**H. densus Sharp**
5. Punctures on head smaller than pronotum (striae deeply grooved and strongly punctures), serial punctures relatively small confluent and forming grooves…….....**H. anchoralis Sharp**
- Puncture on head and pronotum quite strongly closely and regularly punctures, serial punctures deeply grooved and strongly densely puncture…..**H. crenatus Regimbart**

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References

Deepa, J. 2010. Checklist of Aquatic Coleoptera, (Zoological Survey of India, e-publications). www. zsi. Gov.in Sept. 2010.

Hansen, M. 1999. World Catalogue of Insects. Hydrophiloidea (Coleoptera. Appollo Books, Stenstrup vol.2 P. 416.

Hebauer, F. 2001. The real Helochares taprobanicus Sharp 1890 and its allies (Coleoptera: Hydrophiliidae). Latissimus 14:10-16.

Jia Feng-long, Wang jia, Wang Ji-feng, Deng Rui, 2010. Two species of Hydrophilid Beetles (Coleoptera: Hydrophilidae) new to China. Entomotaxonomia 1:33-35.

Komarek, A. 2003. Checklist and Key to Palaearctic and Oriental genera of aquatic Hydrophilidae. Water Beetles of China 3:383-395.

Orchymont, A. d´. 1923. Neue Oder interessante Sphaeridiinen und Hydrophilinen der Malayischen Region [J]. Treubia 3:416-421.

Regimbert, M. 1903. Contribution a la faune Indo-Chinois 19th memoire. Annales de la Societe Entomologique de France 72:52-64.

Sato, M. 1976. Two Helochares species from the Ryukyus (Hydrophilidae). Entomological Review of Japan XXIX (1/2): 21-24.

Sharp, D. 1890. On some aquatic coleopteran from Ceylon. Transaction of the Entomological Society of London 339-359.

Short, A.E.Z. and Hebauer, F. 2006. World Catalogue of Hydrophiloidea – additions and corrections, I (1999-2005). Koleopterologische Rundschau 76:315-359.

Short A.E.Z. and Fikacek, M. 2011. World Catalogue of the Hydrophiloidea (Coleoptera: Additions and Corrections II (2006-2010). Acta Entomologica Musei Nationalis Pragae 51(1): 83-122.

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Additional notes on the genus Apodynerus Giordani Soika, 1993 (Hymenoptera: Vespidae: Eumeninae) from the Indian subcontinent

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Abstract

Apodynerus formosensis indicus Giordani Soika, 1994, is recorded here for the first time from India. Two new synonyms for Apodynerus troglodytes troglodytes (de Saussure, 1856) are proposed, namely, Antepipona malabarica Lambert, 2004, syn. n. and Antepipona narendrani Lambert, 2004, syn. n. The parasitic association of Strepsiptera is reported here for the first time in the genus Apodynerus.

Keywords: Apodynerus, Indian subcontinent, taxonomy, New Synonymy, new record, ethology.

Introduction

Apodynerus is an Oriental genus currently having eleven species with three additional subspecies (Nugroho et al. 2014), of which three taxa, namely Apodynerus formosensis indicus Giordani Soika, 1994, A. icarioides (Bingham, 1897) and A. troglodytes troglodytes (de Saussure, 1856) are recorded from the Indian subcontinent (Girish Kumar et al. 2013). This paper is the continuation of the study by Girish Kumar et al. (2013). We record Apodynerus formosensis indicus Giordani Soika, 1994, for the first time from India. We also examined the holotypes of Antepipona malabarica Lambert, 2004, and Antepipona narendrani Lambert, 2004, and came to the conclusion that both of them are Apodynerus troglodytes troglodytes (de Saussure, 1856). Consequently Antepipona malabarica Lambert, 2004, and Antepipona narendrani Lambert, 2004, are here synonymized under Apodynerus troglodytes troglodytes (de Saussure, 1856). The present paper also reports the parasitic association of Strepsiptera in the genus Apodynerus for the first time.

Material and methods

The materials examined are deposited in the 'National Zoological Collections' of the Hymenoptera Section of the Zoological Survey of India, Kolkata (NZC). The pinned-and-dried specimens were studied and photographed by using a Leica Stereo microscope with LAS software version 3.6.0.

Abbreviations used for the Museums: BMNH = The Natural History Museum, London, UK; MRSN = Museo Regionale di Scienze Naturali di Torino, Italy; NZC = Zoological Survey of India, Kolkata, India; ZSIC = Western Ghat Regional Centre, Zoological Survey of India, Kozhikode (= Calicut), India.

Abbreviations used for the terms: H = Head; M = Mesosoma; S = Metasomal sternum; T = Metasomal tergum.

Results

Apodynerus formosensis indicus Giordani Soika, 1994
(Figs. 1-6)

Apodynerus formosensis indicus Giordani

(Online: 17-November-2014; Print: 9-January-2015)

Soika, 1994: 208 (key), 218, female - "Nepal: Taplejung District, Snagu, 6500 ft" (BMNH).

Diagnosis: **Female** (Fig. 1): Length (H+M+T1+T2) 8.5 mm. Clypeus (Fig. 2) with dense and deep punctures, apex distinctly emarginate, in frontal view convex without carina and in lateral view convex; punctures on frons and vertex more or less dense, slightly smaller and sparser in posterior part of vertex; anterior face of pronotum glossy and punctured laterally, with a few median striae; mesepisternum (Fig. 4) with dense punctures, interspaces non-cariniform; border between lateral face and dorsal face of propodeum ill-defined; submarginal carina of propodeum (Fig. 5) short and wide; T2 in lateral view only slightly convex laterally; baso-median furrow of S2 distinct; punctuation of S2 finer than Apodynerus formosensis formosensis and A. f. continentalis. Body with moderately short setae.

Colour: Body black with yellow and ferruginous markings. Yellow markings as follows: base of mandible; clypeus except for a central black spot; a spot above interantennal space; a strip along the inner orbits of lower lobes of eyes; metanotum almost entirely; T1 with narrow apical band, which is very slightly tapered at sides; bands wider and less regular on T2 and S2. Ferruginous markings as follows: scape; ventral side of funicular segments; a large mark on temple; front half of dorsal face of pronotum, and a narrow band on posterior margin; a mark on top of mesepisternum; a broad band on scutellum; two spots on dorsal side of propodeum; two spots on posterior declivity of propodeum; propodeal valvula; apex of femora, tibiae and tarsi of all legs; tegula; parategula; narrow bands on the apex of T3-T5 and S3-S5. Wings light brown.
Male: Unknown.

Material examined: INDIA: Arunachal Pradesh, Upper Siang district, Geku, 1♀, 16.ix.2002, Coll. Mihir Sil & Party, NZC Regd. No. 15683/H3.

Distribution: India (new record): Arunachal Pradesh; Nepal.

Discussion: In the original description by Giordani Soika (1994) the body is stated to be black with ferruginous markings. But in the specimen studied here from Arunachal Pradesh some markings are yellow instead of ferruginous, such as the marks on clypeus, interantennal space, inner orbits of the lower lobes of the eyes, metanotum, T1, T2 and S2.

Apodynerus troglodytes troglodytes (de Saussure, 1856)

Odynerus troglodytes de Saussure, 1855: 249, male (in subgenus Odynerus division Parodynerus), "Le Sénégal" (MRSN).

Apodynerus troglodytes troglodytes; Gusenleitner, 1988: 180.

Antepipona malabarica Lambert, 2004: 554 (key), 560, ♀, "India, Kerala, Calicut University Campus", holotype ♀ (ZSIC). **Syn. n.**

Antepipona narendrani Lambert, 2004: 558, ♀♂, "India, Kerala. Sulthan Bathery", holotype ♀ (ZSIC). **Syn. n.**

Synonymy of Antepipona malabarica and Antepipona narendrani under Apodynerus troglodytes troglodytes: The holotype of Antepipona malabarica Lambert, 2004, is a female collected on the Calicut University Campus, Kerala, India (Regd. No. ZSI/WGRC/I.R. – INV. 1921; ZSIC 1. 0102) (Figs. 7 & 8). The holotype of Antepipona narendrani Lambert, 2004 is also a female, collected from Sulthan Bathery, Kerala, India (Regd. No. ZSI/WGRC/I.R. – INV. 1920; ZSIC 1. 0101) (Figs. 9 & 10). We examined both holotypes and came to the conclusion that both of them are Apodynerus troglodytes troglodytes (de Saussure, 1856) and thus we synonymize both of them under the latter.

Additional material examined (other than the specimens studied in Girish Kumar et al. 2013): INDIA: Andaman & Nicobar Islands, South Andaman, Ferrargunj, 1♂, 9.xii.2013, Coll. G. Srinivasan & Party, NZC Regd. No. 15686/H3; South Andaman, Ferrargunj, Sonapad, 1♀ & 4♂, 26.xii.2013, Coll. G. Srinivasan & Party, NZC Regd. Nos. 15687/H3–15691/H3; Middle Andaman, Rangat, 1♂, 5.i.2014, Coll. G. Srinivasan & Party,

NZCRegd. No. 15692/H3; North Andaman, Mayabunder, 2♂, 7.i.2014, Coll. G. Srinivasan & Party, NZC Regd. Nos. 15693/H3 & 15694/H3. Kerala, Wayanad district, Chulliyodu, 1♀, 4.x.2014, Coll. P.Girish Kumar, NZC Regd. No. 15695/H3; Kozhikode district

Plate I

Figs. 1–6. *Apodynerus formosensis indicus* Giordani Soika ♀. 1. Body profile; 2. Head frontal view; 3. Head & mesosoma dorsal view; 4. Head & mesosoma lateral view; 5. Propodeum; 6. Metasoma dorsal view.

Plate II

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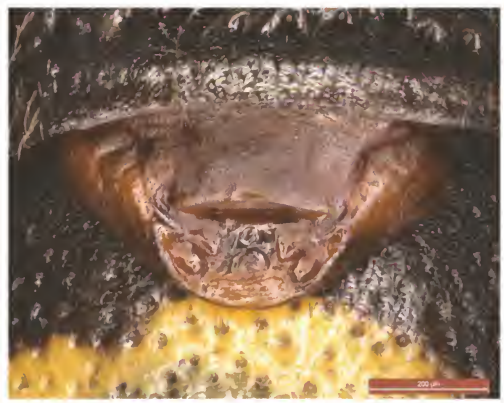

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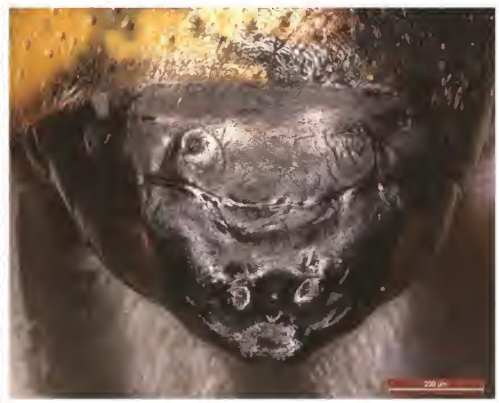

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Figs. 7–8. *Antepipona malabarica* Lambert Holotype. 7. Body profile; 8. Metasoma dorsal view. Figs. 9–10. *Antepipona narendrani* Lambert Holotype. 9. Body profile; 10. Head & mesosoma dorsal view. Figs. 11–12. *Apodynerus troglodytes troglodytes* (de Saussure) metasomal segments with parasitic strepsipteran female.

Nanminda, 2♀ & 2♂, 4.vi.2014 & 6.x.2014, Coll. P. Girish Kumar, NZC Regd. Nos. 15696/H3–15699/H3. West Bengal, Cooch Behar district, Chhatoa, 2♂, 23.xii.2013, Coll. B. Mitra & Party, NZC Regd. Nos. 15700/H3 & 15701/H3.

Distribution: India: Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Karnataka, Kerala, West Bengal. Elsewhere: China (including Hong Kong), Myanmar, Thailand, Laos, Vietnam, Malaysia (including Sarawak, Sabah), Singapore, Indonesia (Sumatra, Krakatau Islands, Kepulauan Seribu, Java, Kangean Islands, Bali, Karimunjawa Islands, Sulawesi, Moluccas, Lombok, Sumbawa, Kalimantan).

Ethology: In this study we observed an interesting behaviour, i.e., parasitic association of strepsipteran insects, for the first time in the genus Apodynerus. Female strepsipteran parasites were observed under the metasomal terga segment of Apodynerus troglodytes troglodytes in one female specimen from South Andaman (Fig. 11) and a male specimen from West Bengal (Fig. 12). The female strepsipteran endoparasites are larviform, leg-less and wing-less, without eyes, antennae and external genitalia. In figures 11 & 12 the female strepsipteran parasites are seen as partially projecting from wasp's metasoma.

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References

Giordani Soika, A. 1994. Ricerche sistematiche su alcuni generi di Eumenidi della Regione Orientale e della Papuasia. Annali del Museo Civico di Storia Naturale "G. Doria" 90: 1-348.

Girish Kumar, P., Mohammed Shareef, K.P., Lambert, K. and Carpenter, J.M. 2013. A taxonomic study on the Oriental genus Apodynerus Giordani Soika (Hymenoptera: Vespidae: Eumeninae) from the Indian subcontinent. Biosystematica 7(1): 23-31.

Gusenleitner, J. 1988. Über Eumenidae aus Thailand, mit einer Bestimmungstabelle fürorientalischer Labus- Arten (Hymenoptera Vespoidea). Linzer Biologische Beiträge 20(1): 173-198.

Lambert, K. 2004. Two new species and a new subspecies of Antepipona Saussure (Hymenoptera: Vespidae) from India with a key to species. In: Rajmohana, K., Sudheer, K., Girish Kumar, P. and Santhosh, S. (eds.). Perspectives on Biosystematics and Biodiversity, Prof. T. C. Narendran Commemoration Volume, University of Calicut, Kerala, India: 553-566.

Nugroho, H., Kojima, J. and Ubaidillah, R. 2014. Synonymy of the potter wasp genus Philippodynerus Gusenleitner (Hymenoptera, Vespidae, Eumeninae) with Apodynerus Giordani Soika, with taxonomic notes on Apodynerus species. Journal of Hymenoptera Research 36: 131-151.

Saussure, H. de. 1854-1856. Études sur la famille des Vespides. Toisième partie comprenant la Monographie des Masariens et un supplement a la Monographie des Euméniens. V. Masson, Paris & J. Kessmann & J. Cherbuliez, Genève, 352 pp. + 15 pls. (1854) 1-48 + pl. 1-5; (1855) 49-288 + pl. 6-14; (1856) 289-352 + pl. 15, 16. [Dates of publication after Griffin 1939].

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A new species of Notanisus Walker (Hymenoptera: Pteromalidae) from Southern Western Ghats, Karnataka, India.

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Abstract

A new species of Pteromalidae, Notanisus elongatus sp. n. is described from Southern Western Ghats of Coorg district, Karnataka. The affinities of new species with related species are discussed.

Keywords: Hymenoptera, Pteromalidae, Notanisus, new species, Coorg, Karnataka, Southern Western Ghat.

Introduction

The genus Notanisus Walker, 1837 belongs to the subfamily Cleonyminae under the tribe Cleonymini of Pteromalidae and is represented by 11 described species and many undescribed species in the old world (Gibson, 2003, Noyes, 2003). The species of Notanisus are tiny parasitoids of either wood-boring coleopterans or hymenopterans developing inside the stems of grasses (Boucek, 1988, Gibson, 2003). Only one species N. versicolor Walker is known from the Oriental Region reported from India (Tamil Nadu, Bihar) (Boucek et al., 1979, Subba Rao & Hayat, 1986). According to Boucek et al., 1979, the report of N. versicolor from India by Saraswat & Mukherjee (1975) appears to be different from that species, though it may belong to the same genus. Since the observation of Boucek et al. 1979 is not based on the study of the specimens, here the report of N. versicolor from India is treated as such. Notanisus species are very rare and random appears in sweep net collections and the present species is described here based on a single female specimen. All efforts to collect further specimens from the area did not yield any additional material due to the rare nature of the genus.

Materials and Methods

The specimen of the present study was collected by sweep net from the forested tracts of Southern Western Ghats falling in the Coorg District of Karnataka, represented by moist deciduous forests. The specimen was card mounted and studied under a stereoscopic binocular microscope (Leica M 205C) and photographs were taken with the camera MC170 HD attached with the microscope. The terminology used in the paper generally follows Boucek (1988) except for the terms mesosoma and metasoma used for thorax and gaster respectively. The type specimen is deposited in the National Zoological collections of Zoological Survey of India, Western Ghat Regional Centre, Calicut (ZSIK). The following abbreviations are used in the text: F1–F5- Funicular segments 1 to 5; MV-Marginal vein; OOL- Ocellocular distance; PMV- Post marginal vein; POL- Post-ocellar distance; SMV- Submarginal vein; STV- Stigmal vein; T1-T5- Gastral tergites 1-5.

Notanisus Walker

Notanisus Walker, 1837: 352. Type species Notanisus versicolor Walker, 1837 by monotypy. For further synonyms refer Gibson (2003).

Diagnosis: Body with metallic lusture. Head without distinct scrobal depression and with inter-antennal and lower parascrobal regions weakly convex. Antennae 13-segmented, formula 11173, inserted at least below the lower eye margin in females, but either below or above lower margin of eyes in males. Female flagellum

(Online: 29- November-2014; Print: 9-January-2015)

with apical margin of the preclaval segment angulate or extending more conspicuously as attenuated fingerlike process along side of clava, clava variably with distinct digit like process apically; male flagellum ramose, with 4-6 long, slender rami. Pronotum elongated, with posterior margins parallel; females macropterous, sometimes micropterous or brachypterous, forewings usually with variable infuscate patterns. Gaster petiolate, the petiole at least subquadrate or longer.

Distribution: Old world, North America (accidentally introduced) (Gibson, 2003).

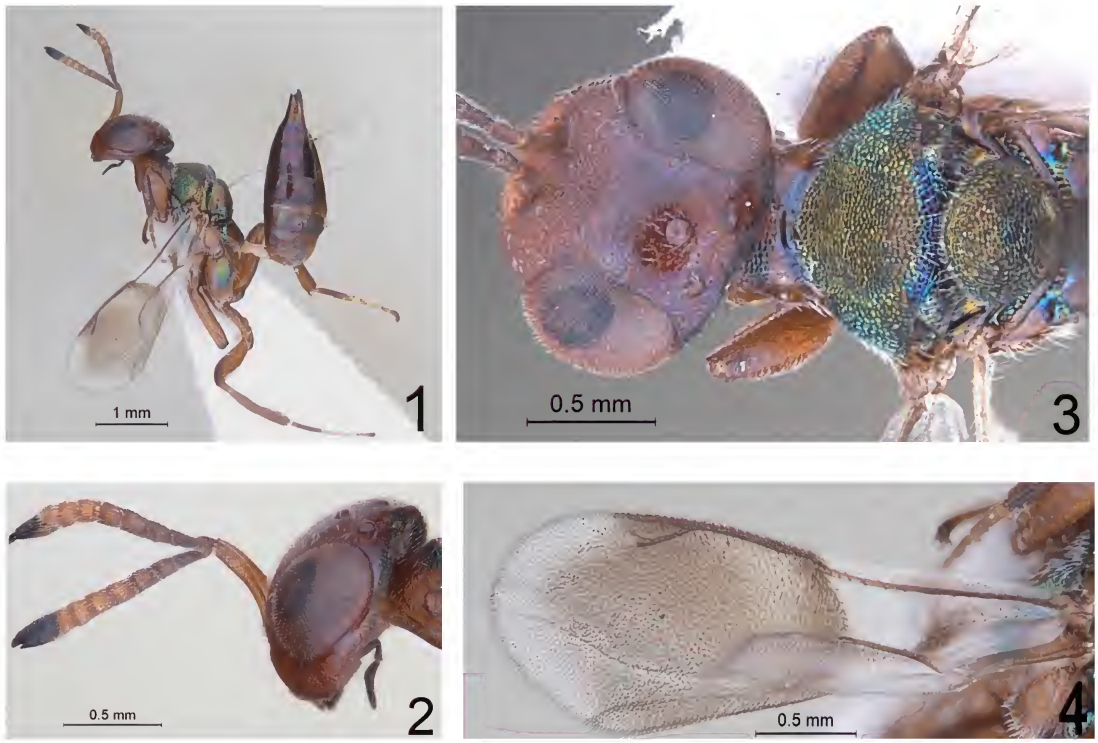
Notanisus elongatus Raseena et Sureshan sp.n.
(Figs.1-4)

urn:lsid:zoobank.org:act:2724AD9F-CF87-47D2-8B7E-D17A135F2A54

Material examined: Holotype: Female, India: Karnataka, Coorg, Brahmagiri Wildlife sanctuary, Mepalli, 2.xi.2013, coll. P. M. Sureshan. Reg.No. ZSI/WGRS/IR/INV/3980.
Description: Female: Length 4.51mm. Body honey brown except for the following: mesoscutum and scutellum completely, dorso-lateral corners of pronotal collar, metanotum and dorsellum dorsally, prepectus, mesopleuron and anterior lateral part of hind coxae bright metallic bluish green with golden refection. Posterior margin of mesoscutum and axillae violaceous. Antennae yellowish brown except terminal part of clava black, scape paler. Eyes cupreous, ocelli brown. Legs yellowish brown. Forewing with two brown infuscations, one broad, below marginal and stigmal veins and other very narrow on cubital setal line. Gaster petiole testaceous, epipygium bluish green.

Head: (Figs. 1, 2, 3) in front view 1.2x as broad as long, in dorsal view 2x as broad as long; malar grooves distinct with a fine ridge at the basal part. Face up to a little above middle distinctly reticulate punctate, pubescence small and white; rest of the face up to posterior margin of vertex broadly engraved reticulate, almost shiny, pubescence sparse; gena moderately reticulate; scrobal area slightly depressed and reticulate; eyes separated 1.5x their length at level of toruli; occiput anteriorly moderately reticulate; rest of the area moderately and transversely reticulate; temple narrow, 0.2 x as long as eye length; POL 2.26x OOL. Antenna (Fig.2) inserted slightly below lower margin of eyes, toruli separated 2.1x their individual diameter; inter antennal area raised; scape 0.85x as long as eye and slightly swollen towards the tip, pedicel plus flagellum 0.91x head width, pedicel 1.3x as long as broad, anellus 0.3x F1, 1.43x as long as broad. Relative lengths of F1 6, F2 7, F3 8, F4 7, F5 7, F6 5.5, F7 5, clava 15.

Mesosoma: (Figs. 1, 3) Pronotum as long as broad and 0.58 less wider than mesoscutum, collar distinctly reticulate in the anterior half and shiny in posterior half. Mesoscutum 1.71x as broad as long, uniformly punctuate reticulate, pubescence white, notauli incomplete; axilla moderately reticulate in anterior part with transverse rugae in posterior part. Scutellum little wider than long, reticulate punctuate, shiny towards posterior end, frenum not separated. Dorsellum broad and shiny. Propodeum 1.61x as broad as median length. 0.6x as long as scutellum medially, smooth , median carina distinct and complete, anterior, posterior and lateral margins and area of median carina with distinct crenulated groove separated by longitudinal rugae , plical groove with similar groove separated by longitudinal rugae, spiracles large and oval, separated by own diameter from the hind margin of metanotum, callus shiny with scattered long hairs. Mesopleuron distinctly reticulate except upper mesepimeron shiny. Metapleuron almost shiny. Forewing (Fig.3) 3.04x as long as broad ,basal half partly bare, costal cell with a row of hairs in the middle, speculum present, basal hair line not indicated, cubital hair line with numerous setae at tip, basal cell bare, discal pubescence dense with hairs in the form of setae. Relative lengths SMV 4.7, MV 2.7, PMV 1.5, STV 1.2. Hind coxae 1.4x as long as broad, distinctly reticulate,



Figs.1-4. Notanisus elongatus Raseena & Sureshan sp.n. Female: 1. Body profile view; 2. Head in profile view; 3.Head and mesosoma in dorsal view; 4. Forewing.

punctate dorso-laterally, femur engraved reticulate, hind part smooth, hind tibia with two spurs.

Metasoma: (Fig.1) Petiole 2x as long as broad, shiny, with a pair of longitudinal rugae laterally, gaster 2.6x as long as broad in dorsal view and slightly shorter than head plus mesosoma combined (0.95x), posterior margin of tergites straight, T1 with 3-4 hairs on dorso-lateral sides; T4 & T5 largest and equal. T3 almost half of T4 dorsally, T3 onwards finely reticulate dorsally, reticulation on epipygium distinct.

Comments: This species resembles N. versicolor Walker, 1937 in some general morphology but differs in having body not slender with pronotum short, forewing with different pattern of infuscations, one broad below MV, STV and PMV and other small on cubital setal line, gastral petiole long, 2x as long as broad, scutellum distinctly reticulate with bottom of individual areolae microreticulate, propodeum shiny with distinct crenulated groove on median area, anterior, posterior and lateral margins separated by distinct rugae and antennae with anelli almost quadrate (in N. versicolor, body more slender with pronotum longer, forewing with different infuscations, there are two, one broad below STV and one narrow below parastigma both separated by a clear hyaline band, gastral petiole about 1.5x as long as broad, scutellum reticulate, bottom of individual areolae shiny, propodeum except the median crenulated double groove smooth).

Etymology: The species name is derived from the elongate gastral petiole.

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References

Boucek, Z. 1988. Australasian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of fourteen families, with a reclassification of species. CAB International Wallingford: 832 pp.

Boucek, Z., Subba Rao, B.R. and Farooqi, S.I. 1979. A preliminary review of Pteromalidae (Hymenoptera) of India and adjacent countries. Oriental Insects 12(4): 433-468.

Farooqi, S.I. and Subba Rao, B.R. 1986. Family Pteromalidae. (In: Subba Rao, B.R.; Hayat, M. (Eds)- The Chalcidoidea (Insecta: Hymenoptera) of India and the adjacent countries. Part II.A catalogue. Oriental Insects 20:295.

Gibson, G.A.P. 2003. Phylogenetics and classification of Cleonyminae (Hymenoptera : Chalcidoidea : Pteromaliadae). Memoirs on entomology, International. USA. 339 pp.

Noyes, J.S. 2003. Universal Chalcidoidea Database. World Wide Web electronic publication http://www.nhm.ac.uk/chalcidoids. Last updated May 2014. (Accessed in September 2014).

Saraswat, G.G. and Mukherji, M.K. 1975. Records of some known and descriptions of some new species of chalcids (Hymen-optera).Memoirs on School of Entomology, St.John's College Agra 4:35-62.

Walker, F. 1837. Monographia Chalciditum. Entomological Magazine 4: 349-364.

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Mosquito species biodiversity in Phytotelmata from Western Ghats, south India

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Abstract

The Western Ghats hill ranges in Indian peninsula are one of the 25 hot spots in the world. Western Ghats hill ranges are rich with fauna and flora. This study was carried out in 11 hill ranges of Western Ghats falling in three States of South India, by the Centre for Research in Medical Entomology (CRME) Madurai, during July 2010 to October 2013. Altitude of the hill ranges span between 300m-2300m which receives rains from both southwest and northeast monsoons. Major emphasis was given to survey immatures in Phytotelmata habitats. 124 mosquito species were recorded belonging to 30 genera and 24 subgenera in 11 Phytotelmata habitats. Among these 10 Anopheles and 114 Culicine species were recorded. One malaria vector (Anopheles culicifacies), two JE vectors (Culex pseudovishnui, Cx. whitmorei) and two dengue/chikungunya vectors (Stegomyia aegypti, St. albopicta) were recorded.

Keywords: Phytotelmata, Western Ghats, tree holes, mosquitoes.

Introduction

A term Phytotelmata ('plant-waters') was coined by Varga (1928) to describe bodies of water impounded by plants. According to Fish (1983) over 1500 different plant species from at least 26 families have been reported to impound water. Leaf axils seem to be the most common type of Phytotelmata (Greeney, 2001), with the Bromeliaceae alone believed to have more than 1000 species capable of impounding water (Frank, 1983). Phytotelmata can be seen as aquatic microcosms since, despite the very small volume of rainwater collected in them, micro-communities have been found there which, relative to the size of the habitat have a very diverse range of taxa, which survive and interact as small ecosystems. Around 470 species of aquatic animals have been recorded so far in these bromeliad tanks. The literature on mosquitoes inhabiting phytotelmata is relatively extensive, particularly due to bromeliad-breeding species and their public health significance in tropical regions (Olano et al. 1997; Forattini et al. 1998; Cunha et al. 2002). Christopher (1933) and Barraud (1934) in Fauna of British India (Family Culicidae), identified more than 25 phytotelmata habitats and recorded 222 mosquito species. Two-letter generic and three-letter sub generic abbreviations used in the paper were adopted from Reinert (1975).

Material and Methods

Study area

Eleven hill ranges were surveyed in Western Ghats, comprising three states viz; Agastya hills, Andipatti hills, Varusanad hills, Palani hills, Anamalai hills, Nilgiri hills (Tamil Nadu), Munnar, Malappuram, Parambikulam and Silent valley (Kerala), Coorg (Karnataka), from 2010 to 2013. Altitude of the hill ranges ranged between 300m-2300m which receives rain from both southwest and northeast monsoons. Major emphasis was given to surveys, for the collection of larval and pupal stages of mosquitoes which breed in phytotelmata habitats such as tree hole, log hole, bamboo stump, bamboo sheath, fallen tree, papaya stump, reed stumps, leaf axils, banana stump, mushroom, pitcher plant. Identification of species was based mainly on adult characters, however larval and pupal chaetotaxy were also examined wherever necessary (Tyagi et al. 2012, 2014).

(Online: 30-December-2014; Print: 9-January-2015)

Results and Discussion

A total of 124 mosquito species were recorded from 36044 specimens belonging to 30 genera and 24 subgenera in 11 Phytotelmata habitats (Table 1). Tree holes were the most favorable habitat, which contributed 99 species from 21440 specimens followed by bamboo stumps 73 species from 11753 specimens, log hole 20 species from 637 specimens, leaf axils 10 species from 561 specimens etc., (Fig. 1). Among these 10 Anopheles and 114 Culicine species were recorded. One malaria vector (Anopheles culicifacies) was collected from typical wide open hole (25-50 cm. diameter) near the base of felled tree trunks (fallen tree), two Japanese encephalitis vectors (Culex pseudovishnui, Cx. whitmorei) were recorded from tree holes and bamboo stumps and two dengue/chikungunya vectors (Stegomyia aegypti, St. albopicta) were recorded from tree holes, bamboo stumps, reed stumps and log holes. Stegomyia albopicta (5070) was numerically the most abundant species and also recorded from six habitats followed by Tripteroides aranoides (3247), Dendroskusea reginae (2623), Orthopodomyia flavithorax (2280) and Armigeres subalbatus (2268).

Notes on few important Phytotelmata habitats

Tree hole and Log hole

Tree holes are the primary breeding sites for multiple disease vectors, most frequently mosquitoes (Yanoviak 2001). No study has explicitly examined tree hole occurrence according to specific genus or species of tree, tree-level factors such as diameter at breast height and stand-level characteristic such as basal area. Mattingly (1969) classified tree holes according to their means of saturation into two distinct groups. One group of tree holes are filled constantly with water drawn up by the roots of the tree, and other group is dependent on rainwater. Because the tree hole community is based on the leaves that fall into them, these communities are often called detritus-based communities. In Western Ghats maximum number of mosquito species (99) were recorded in tree holes (Figs. 2, 3).

However, very few studies have addressed water chemistry factors that are potentially limited to tree hole insect populations (Petersen and Chapman, 1969; Mitchell and Rockett, 1981; Beier et al. 1983; Paradise, 1979). Mosquitoes inhabiting tree holes show increased mortality, decreased growth rates and smaller size when stem flow contains high concentrations of hydrogen ions (Carpenter, 1982). Nutrients, including sulfates and nitrates, can also adversely affect mosquito populations in tree holes, in part by changing bacterial population dynamics (Walker et al. 1991).Most of these arthropods are obligate tree hole breeders, although they also utilize other small bodies of standing water in man-made containers that simulate tree holes (Laird, 1988). The size and location of the tree hole may be important in determining presence/absence of any particular species.

Bamboo stumps

Different types of bamboo are found in the rich and biodiverse regions of Western Ghats. Water tight containers are formed by the wood-chewing beetles in the internodes of the bamboo stem. Once the beetles get inside of the internodes they lay their eggs. When the eggs hatch, the larvae of beetles chews on the plant tissue and make their way out of the bamboo internodes leaving a bigger hole. Once these holes have been formed a phytotelmata habitat gets created filled with water ready for different species to inhabit it. During present investigations, about 73 mosquito species were recorded in these habitats (Figs. 4, 5).

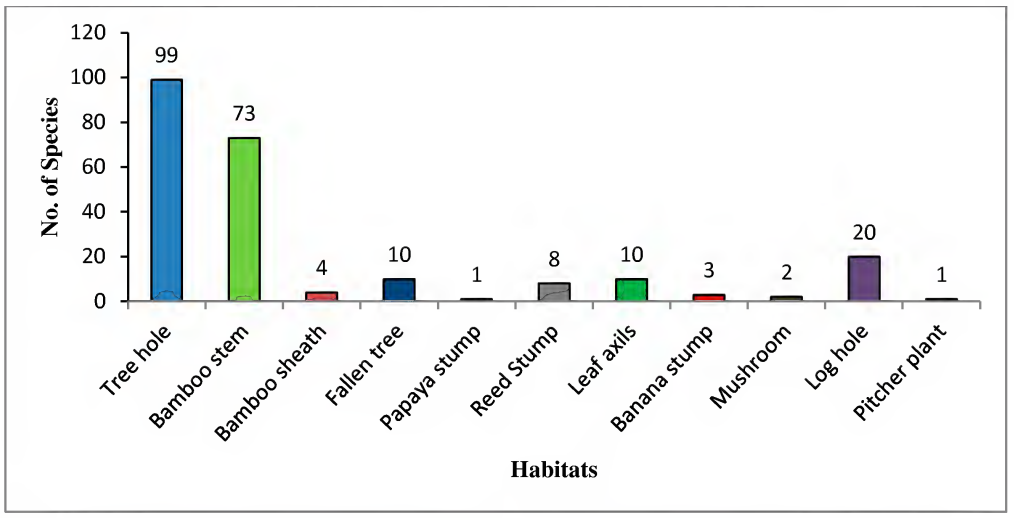
Plant leaf axils

Leaf axil habitats are formed when water fills up in the axil of leaves. Axil habitats are small and hold a simple aquatic community comprising mainly of filter-feeders. Mosquitoes breed in leaf axils of many plants like Plantain, Pineapple, Pandanus, Nipa, Taro, and Colocasia. In Western Ghats 10 mosquito species were recorded from leaf axils (Figs. 6, 7).

Pitcher plants

Pitcher plants with their water tight containers act as traps for insects and other small creatures. The detritus created by dead decomposing insects inside the pitcher creates an organically rich aquatic environment for the pitcher tank biotic community. A total of fifteen mosquito species representing three genera Aedes, Wyeomyia and Toxorhynchites

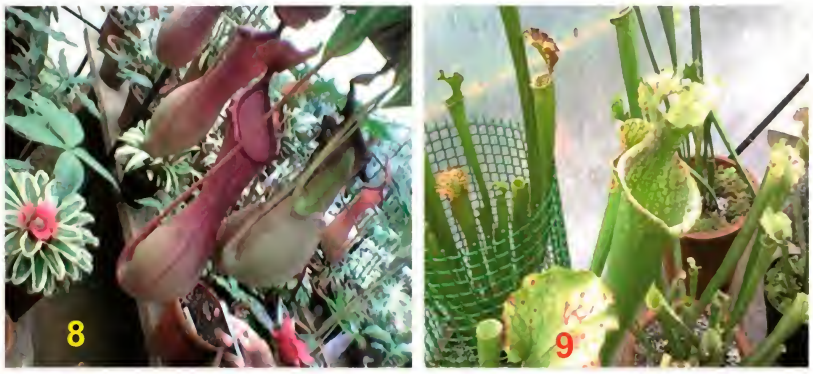
Figure 1. Habitat wise distribution of mosquito species in Western Ghats



Figs. 2, 3: 2. Tree hole; 3. Log hole

Figs. 4, 5: 4. Bamboo stump; 5. Bamboo fence

Figs. 6,7: 6. *Colocasia*; 7. Pineapple



Figs. 8, 9: *Nepenthes sp.*

Table 1. Mosquito species recorded (through Immature collection) from phytotelmata habitats in Western Ghats

Sl. No.	Species/Breeding habitat	Habitat											No. of Specimen
		Tree hole	Bamboo stump	Bamboo sheath	Fallen tree	Papaya stump	Reed stump	Leaf axils	Banana stump	Mushroom	Log hole	Pitcher plant	
1	Anopheles (Anopheles) aitkenii	**5**											**5**
2	Anopheles (Anopheles) culiciformis	**2**											**2**
3	Anopheles (Anopheles) insulaeflorum	**3**											**3**
4	Anopheles (Anopheles) interruptus	**28**											**28**
5	Anopheles (Anopheles) sintoni	**148**											**148**
6	Anopheles (Cellia) culicifacies				**99**								**99**
7	Anopheles (Cellia) elegans	**8**											**8**
8	Anopheles (Cellia) mirans	**63**	**5**								**3**		**71**
9	Anopheles (Cellia) jeyporiensis	**7**											**7**
10	Anopheles (Cellia) maculatus	**3**											**3**
11	Aedimorphus alboscutellatus		1										**1**
12	Aedimorphus caecus	21	54								9		**84**
13	Armigeres (Armigeres) durhami		350			36							**386**

14	Armigeres (Armigeres) joloensis	4											**4**
15	Armigeres (Armigeres) subalbatus	1535	417				73	30	119			94	**2268**
16	Armigeres (Leicesteria) digitatus		39										**39**
17	Armigeres (Leicesteria) flavus	19	956	28			96						**1099**
18	Armigeres (Leicesteria) longipalpis		3										**3**
19	Armigeres (Leicesteria) magnus		22										**22**
20	Armigeres (Leicesteria) omissus	4	76										**80**
21	Bruceharrisonius aureostriatus	347	78		6								**431**
22	Bruceharrisonius greenii	5						28					**33**
23	Christophersiomyia annulirostris	344	1								13		**358**
24	Christophersiomyia gombakensis	1											**1**
25	Christophersiomyia thomsoni	5											**5**
26	Collessius (Alloeomyia) pseudotaeniatus	166	3								29		**198**
27	Culex (Culex) mimiticus	41											**41**
28	Culex (Culex) mimulus		3										**3**
29	Culex (Culex) niligricus	5									2		**7**
30	Culex (Culex) pseudovishnui	3											**3**
31	Culex (Culex) whitmorei		1										**1**
32	Culex (Culiciomyia) bailyi	2											**2**
33	Culex (Culiciomyia) fragilis	13	10										**23**
34	Culex (Culiciomyia) nigropunctatus		3										**3**
35	Culex (Culiciomyia) pallidothorax	100	106		3				5		6		**220**
36	Culex (Culiciomyia) spathifurca	171											**171**
37	Culex (Eumelanomyia) brevipalpis	864	29										**893**
38	Culex (Eumelanomyia) khazani	236	1										**237**
39	Culex (Eumelanomyia) malayi		3										**3**
40	Culex (Lopoceraomyia) bengalensis	1	3										**4**
41	Culex (Lopoceraomyia) flavicornis	120											**120**
42	Culex (Lopoceraomyia) lasiopalpis	1											**1**
43	Culex (Lopoceraomyia) mammilifer	99	2										**101**
44	Culex (Lopoceraomyia) minor	303	298	2				8			134		**745**
45	Culex (Lopoceraomyia) minutissimus	10											**10**
46	Culex (Lopoceraomyia) peytoni	1											**1**
47	Culex (Lopoceraomyia) pholeter	2	1										**3**
48	Culex (Lopoceraomyia) raghavanii										32		**32**
49	Culex (Lopoceraomyia) uniformis	3471	976		78		15				204		**4744**
50	Danielsia albotaeniata	11	5										**16**
51	Dendroskusea kanarensis	6											**6**
52	Dendroskusea reginae	2623											**2623**
53	Downsiomyia albolateralis	502	151										**653**
54	Downsiomya nivea	429	8										**437**
55	Ficalbia minima	1											**1**
56	Fredwardsius vittatus	451	10					1					**462**
57	Heizmannia (Heizmannia) chandi	104	108										**212**
58	Heizmannia (Heizmannia) greenii	66	21										**87**
59	Heizmannia (Heizmannia) indica	20	51								8		**79**

60	Heizmannia (Heizmannia) metallica	17	25								8		**50**
61	Heizmannia (Mattinglyia) discrepans	98	168				8						**274**
62	Hopkinsins (Yamada) albocinctus	2											**2**
63	Hulecoeteomyia chrysolineata	273	495	13				140	4	68	2		**995**
64	Hulecoeteomyia pallirostris		1					95					**96**
65	Hulecoeteomyia harveyi		10										**10**
66	Kenknightia dissimilis	108											**108**
67	Lorrainea fumida	3											**3**
68	Lutzia (Metalutzia) fuscana	68											**68**
69	Lutzia (Metalutzia) halifaxii	1											**1**
70	Malaya genurostris							164					**164**
71	Malaya jacobsoni							65					**65**
72	Mimomyia (Etorleptimyia) luzonensis		1										**1**
73	Orthopodomyia albipes		18										**18**
74	Orthopodomyia anopheloides	292	8		2						2		**304**
75	Orthopodomyia flavithorax	2280											**2280**
76	Orthopodomyia flavicosta	41											**41**
77	Phagomyia cacharana	3	1										**4**
78	Phagomyia cogilli	4	1										**5**
79	Phagomyia deccana	3											**3**
80	Phagomyia gubernatoris	44	19										**63**
81	Phagomyia inquinata	3											**3**
82	Phagomyia khazani	17											**17**
83	Phagomyia prominens	20	18										**38**
84	Rhinoskusea portonovoensis	19											**19**
85	Scutomyia albolineata	2	3										**5**
86	Stegomyia (Actinothrix) edwardsi	160											**160**
87	Stegomyia (Heteraspidion) annandalei	1	13										**14**
88	Stegomyia (Heteraspidion) craggi	8	16										**24**
89	Stegomyia (Huangmyia) perplexa		21										**21**
90	Stegomyia (Stegomyia) aegypti	119	4				1				2		**126**
91	Stegomyia albopicta	2316	2142		463		3	18			128		**5070**
92	Stegomyia flavopicta	1											**1**
93	Stegomyia krombeini	505	40		242						6		**793**
94	Stegomyia malayensis	30											**30**
95	Stegomyia mediopunctata		8										**8**
96	Stegomyia novalbopicta	483	167										**650**
97	Stegomyia pseudoalbopicta	82	73										**155**
98	Stegomyia subalbopicta	355	38		2								**395**
99	Stegomyia w-alba	2	8										**10**
100	Tewarius agastyai	354	331										**685**
101	Tewarius reubenae	231											**231**
102	Toxorhynchites (Toxorhynchites) edwardsi	5											**5**
103	Toxorhynchites (Toxorhynchites) gravelyi		3										**3**
104	Toxorhynchites (Toxorhynchites) kempi		6										**6**

105	Toxorhynchites (Toxorhynchites) minimus	3	3				1						**7**
106	Toxorhynchites (Toxorhynchites) splendens	250	265		4			12			6		**537**
107	Tripteroides (Rachionotomyia) affinis	533	93								25		**651**
108	Tripteroides (Rachionotomyia)aranoides	150	2951				130				16		**3247**
109	Tripteroides (Rachionotomyia) serratus	4											**4**
110	Tripteroides (Tripteroides) indicus	11											**11**
111	Tripteroides (Tripteroides) tarsalis		13										**13**
112	Uranotaenia (Pseudoficalbia) bicolor	35	950										**985**
113	Uranotaenia (Pseudoficalbia) lutescens	1	22										**23**
114	Uranotaenia (Pseudoficalbia) novobscura	52	4								2		**58**
115	Uranotaenia (Pseudoficalbia) obscura	5			3					54			**62**
116	Uranotaenia (Pseudoficalbia) ohamai	3											**3**
117	Uranotaenia (Pseudoficalbia) recondita	50	6	1									**57**
118	Uranotaenia (Pseudoficalbia) stricklandi	4	1										**5**
119	Uranotaenia (Pseudoficalbia) luteola		5										**5**
120	Uranotaenia (Uranotaenia) annandalei	4											**4**
121	Uranotaenia (Uranotaenia) campestris	4											**4**
122	Uranotaenia (Uranotaenia) edwardsi		6										**6**
123	Uranotaenia (Uranotaenia) orientalis		1										**1**
124	Verrallina (Verrallina) dux	7											**7**
	No. of species recorded in habitats	**99**	**73**	**4**	**10**	**1**	**8**	**10**	**3**	**2**	**20**	**1**	

were recorded from Sarracenia sp. The mosquito Wyeomyia smithii strictly depends on Sarrecenia purpurea to complete its larval development (Bradshaw, 1983). In the food web of the pitcher, Wyeomyia serves as the top predator; the population size of Wyeomyia is negatively correlated with inquiline diversity (Buckley et al. 2003). Nine mosquito species were recorded from India, but in Western Ghats we recorded only one species (Ar. subalbatus) in this habitat (Figs.8, 9).

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References

Barraud, P.J. 1934. The fauna of British India, including Ceylon and Burma. Diptera: Family Culicidae; Tribes Megarhinini and Culicini. London: Taylor & Francis. 5: 463pp.

Beier, J.C., Patricoski, C., Travis, M and Kranzfelder, J. 1983. Influence of water chemical and environmental parameters on larval mosquito dynamics in tires. Environmental Entomology 12: 434-438.

Bradshaw, W.E. 1983. Interaction between the mosquito Wyeomyia smithii, the midge Metriocnemus knabi, and their carnivorous host Sarracenia purpurea. In: Frank JH,

Lounibos LP. eds. Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Medford: Plexus Publishing. 161–189.

Buckley, J.L., Miller, T.E., Ellison, A.M and Gotelli, N.J. 2003. Reverse latitudinal trends in species richness of pitcher-plant food webs. Ecology Letters 6: 825–829.

Carpenter, S.R. 1982. Stemflow chemistry: effects on population dynamics of detrivorous mosquitoes in tree-hole ecosystems. Oecologia 53: 1– 6.

Christophers, S.R. 1933. The fauna of British India including Ceylon and Burma, Diptera, Family Culicidae, Tribe Anophelini. London: Taylor and Francis. 4: 371pp.

Cunha, S.P., Alves, J.R.C., Lima, M.M., Duarte, J.R., Barros, L.C.V., Silva, J.L., Gammaro, A.T., Filho, O.S.M and Wanzeler, A.R. 2002. Presença de Aedes aegypti em Bromeliaceae e depósitos com plantas no município do Rio de Janeiro, RJ. Revista de Saúde Pública 36(2): 244-245.

Fish, D. 1983. Phytotelmata: flora and fauna. In Frank JH. and Lounibos LP. (Eds.). Phytotelmata: terrestrial plants as host for aquatic insect communities. New Jersey: Plexus. 293 pp.

Forattini, O.P., Alvarenga, G.R., Marques, M., Kakitani, I., Brito, M. and Sallum M.A.M. 1998. Significado epidemiológico dos criadouros de Aedes albopictus em bromélias. Revista de Saúde Pública 32 (2): 523-530.

Frank, J.H. 1983. Bromeliad phytotelmata and their biota, especially mosquitoes. In: Frank, J.H.; Lounibos, L.P. (Editors), Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Medford, U.S.A.: 101-128pp.

Greeney, H.F. 2001. The insects of plant-held waters: a review and bibliography. Journal of Tropical Ecology 17: 241-260.

Laird, M. 1988. The Natural History of Larval Mosquito Habitats. London:Academic Press. 535 pp.

Mattingly, P.F. 1969. The Biology of Mosquitoes Borne Disease. London: George Allen and Unwin. 185pp.

Mitchell, L. and Rockett, C.L. 1981. An investigation on the larval habitat of five species of tree-hole breeding mosquitoes (Diptera: Culicidae). The Great Lakes Entomologist 14: 123-129.

Olano, V., Carrasquilla, G. and Méndez, F. 1997. Transmisión de la malaria urbana en Buenaventura, Colombia: aspectos entomológicos. Revista Panamerica de Salud Publica 1: 287-294.

Paradise, C.J. 1998. Colonization and development of insects in simulated tree hole habitats with distinct resource and pH regimes. Ecoscience 5:39-45.

Petersen, J.J. and Chapman, H.C. 1969. Chemical factors of water in tree holes and related breeding of mosquitoes. Mosquito News 29: 29-35.

Reinert, J.F. 1975. Mosquito generic and subgeneric abbreviations (Diptera: Culicidae) Mosquito Systematics 7: 105-110.

Tyagi, B.K., Munirathinam, A., Krishnamoorthy, R. and Venkatesh, A. 2012. A field-based handbook of identification keys to mosquitoes of public health importance in India. Volume 1; Taxonomy and Biodiversity Cell. Centre for Research in Medical Entomology. 55pp.

Tyagi, B.K., Munirathinam, A., Krishnamoorthi, R., Baskaran, G., Venkatesh, A., Kalimuthu, M. and Govindarajan R. 2014. A field-based handbook of identification keys to 4th instar larvae of mosquitoes of public health importance in India. Volume 2; Taxonomy and Biodiversity Cell. Centre for Research in Medical Entomology. 45pp.

Varga, L. 1928. Ein interessanter Biotop der Bioconöse von Wasserorganismen. Biologisches Zentralblatt 48: 143-162.

Walker, E.D., Lawson, D.L., Merritt, R.W., Morgan, W.T. and Klug, M.J. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. Ecology 72:1529-46.

Yanoviak, S.P. 2001. Predation, resource availability, and community structure in Neotropical water-filled tree holes. Oecologia 126: 125–133.

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Second Vietnamese species of the myrmicine genus Lophomyrmex (Hymenoptera, Formicidae)

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Abstract

Lophomyrmex indosinensis sp. n. is described from southern Vietnam. It is distinguished from the related species of the L. bedoti group by the combination of the following characteristics: antennal scape long (SI >100), number of ommatidia along long axis of eye 8-9; vertex and temple very superficially coriaceus and strongly shiny; dorsal disc of pronotum with two or more standing hairs near anterior margin; lateral face of pronotum nearly smooth and shiny; with propodeum in profile posterior margin with several suberect hairs; propodeal spine more or less distinctly upward-directed with its apex distinctly higher than propodeal dorsum.

Keywords: Lophomyrmex, L. bedoti group, new species, Vietnam, nest site.

Introduction

The Oriental and Indo-Australian ant genus Lophomyrmex Emery, 1892 was revised by Rigato (1994), who recognized ten species in two species groups. In the L. bedoti Emery group the pronotal disc is laterally margined with irregular carinae, while in the L. quadrispinosus (Jerdon) group it bears a pair of lateral spines. Sheela and Ghosh (2008) and Bharti and Kumar (2012) added two species from India, both belonging to the L. bedoti group. Most species live on the ground level of primary and secondary forests, and nests in soil or decayed wood. Foragers are frequently collected from leaf litter and attracted to sugar baits (Eguchi and Yamane, 2003). Lophomyrmex longicornis Rigato, a Bornean endemic, actively forages in both the daytime and nighttime (Yamane et al. 1996).

Four species have been known from Thailand (Jaitrong and Nabhitabhata, 2005). However, in Vietnam only one species, L. birmanus Emery, 1893 belonging to the L. quadrispinosus group, has been recorded from central and southern regions (Eguchi et al. 2011). One of the authors, SH, collected an additional species belonging to the L. bedoti group from leaf litter in Southern Vietnam. It is described here as a new species.

Materials and Methods

The workers examined were collected from two colonies in a relatively undisturbed secondary forest with hand sieving of leaf litter under decayed wood.

For measurements and indices we principally follow Bolton (1987) and Rigato (1994) as follows:

Eye length: maximum length of eye.

Eye width: maximum width of eye with head in profile but in a slightly oblique position.

Head length: length of head capsule excluding mandibles measured in full-face view in straight line from mid-point of anterior margin of clypeus to midpoint of posterior margin of head (very slight emargination of posterior margin of head can be ignored).

Head width: maximum width of head excluding eyes.

Scape length: maximum straight-line length of scape excluding basal constriction.

Propodeal spine length: with spine in full lateral view, straight dorsal distance from base of spine to apex; base of spine is defined as mid-point of

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concavity where propodeal dorsum and spine meet.
Hind tibial length: length of hind tibia excluding 'knee' that is concealed by apex of femur when leg is fully straightened.
Images were taken using a Canon EOS 50D with a Canon MP-E 65 mm 1-5 x macro lens, then processed using Combine ZM.

Lophomyrmex indosinensis Yamane et Hosoishi, sp. n. (Figs. 1-3)

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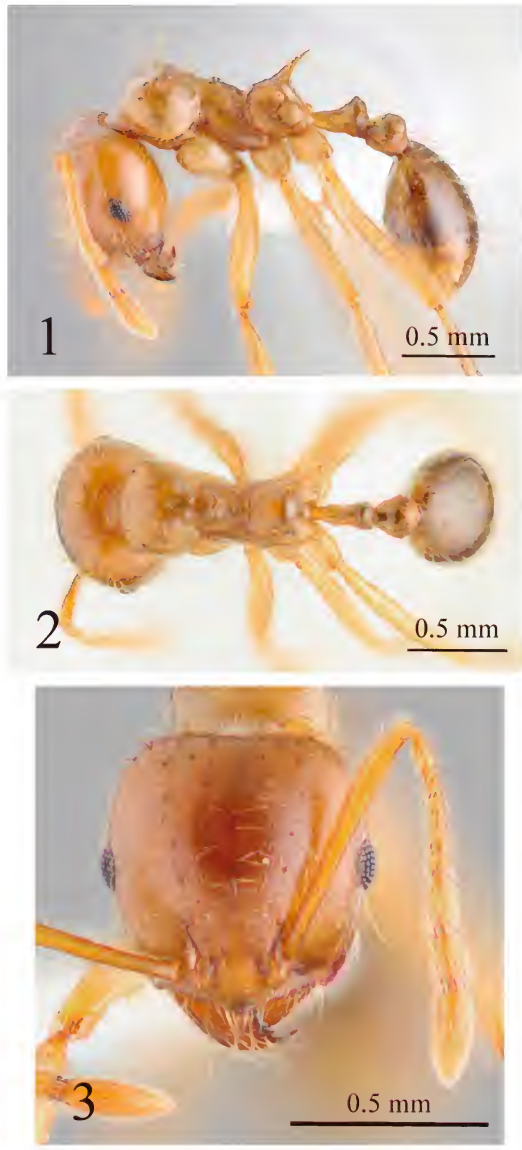
Worker description. Measurements (in mm; n=5; mean in parentheses). Head width 0.72-0.76 (0.74), Head length 0.76-0.80 (0.78), Eye length (EL) 0.15-0.16 (0.16), Eye width (EW) 0.10-0.11 (0.10), Scape length (SL) 0.74-0.76 (0.75), Propodeal spine length 0.21-0.26 (0.24), Hind tibial length (0.70-0.74 (0.73). Cephalic index (CI) 93-97 (95), Scape index (SI) 100-103 (102), EL/EW 1.45-1.56 (1.51).
Head slightly longer than broad, with straight to very weakly convex posterior margin in profile. Clypeus with anterior margin bearing median process. Mandible with large apical tooth followed by smaller second tooth, low triangular third tooth, and several small denticles of variable size. Eye longer than broad but not very elongate, anteriorly not pointed, with 8-9 ommatidia along long axis. Antennal scape relatively long, extending beyond posterior margin of head by much more than length of antennal pedicel (second segment). Pronotum laterally with small tubercle (often with another smaller one); short carina(e) may exists around tubercles but generally weakly developed. In profile mesonotum more or less distinctly elevated at 2/5 length of its slope so that anterior concavity can be seen just behind pronotum. Propodeum with weakly convex dorsal outline; lateral carina on declivity weak, not darkened; propodeal spines nearly as long as propodeal height, diverging with apical portion weakly curved outwardly, obliquely upward-directed with apex distinctly higher than propodeal dorsum. With waist in profile, dorsal outline of petiole shallowly concave, peduncle not distinctly demarcated from node; node apically round or at most with indistinct anterodorsal angle; postpetiole globular with evenly curved dorsal outline, nearly as long as high.
Head weakly and superficially sculptured; area between eye and clypeus longitudinally striate; frontal lobe rather strongly sculptured; temple behind eye and venter of head almost smooth and strongly shiny. Clypeus finely transversely



Figures 1-3. Lophomyrmex indosinensis. 1, Lateral view of body; 2, Dorsal view of body; 3, Full-face view of head.

striate mixed with superficial microreticulation except in basal and apical smooth portions. Mandible densely covered with longitudinal striae and mat except for narrow shiny belt along basal and masticatory margins. Pronotum entirely superficially sculptured and shiny; mesothrax, metapleuron and propodeum densely sculptured except for propodeal declivity rather smooth and shiny; mesopleuron more regularly punctate than other parts and interspaces smooth and shiny. Waist extensively sculptured; anterior and dorsal faces of postpetiolar node more or less smooth. Legs superficially sculptured to smooth except for coxae where sculpture is stronger (fore coxa with much weaker sculpture). Dorsum of head with many long standing hairs; hairs on lateral and ventral faces of head tend to be decumbent. Suberect hairs on antennal scape dense and long, some of which are longer than scape width. Pronotum in frontal view with a pair of anterior erect hairs located at short distance from lateral margin and often additional hairs located more mesad (some of these hairs may be missing); mesonotum with two pairs of short but stiff erect hairs; propodeal dorsum without long erect hairs; propodeal declivity in profile with several short and soft hairs.

Body concolorous brown to dark brown; in paler individuals mesosoma yellowish brown, and head and gaster dark; mandible reddish brown; antenna and legs paler than mesosoma.

Remarks. The specific differences between the species of the L. bedoti group are often very slight. It is highly recommended when identifying species to use series of specimens from colonies. The present new species is similar to L. longicornis Rigato, endemic to Borneo, in having a long antennal scape and weak body sculpture. However, in L. longicornis, the hairs on the antennal scape are nearly appressed, the pair of anterior erect hairs on the pronotum are absent, the hairs of the mesonotal dorsum are short, soft and oblique, the propodeal declivity has a pair of complete lateral carinae that are darkened, the posterior margin of the propodeum bears no short hairs, and the propodeal spine is longer, measuring 0.35-0.39 mm (0.21-0.26 mm in L. indosinensis).

Lophomyrmex bedoti Emery (Borneo to Southern Thailand) and L. striatulus Rigato (Thailand) are also related to L. indosinensis, but these species can be separated by the following differences:

L. indosinensis: CI 93-97 (95), SL 0.74-0.76 mm (0.75 mm), SI 100-103 (102). Eye weakly narrowed anteriad, with anterior apex round, EL 0.15-0.16 mm (0.16 mm), number of ommatidia along long axis 8-9, EL/EW 1.46-1.56 (1.51). Area between antennal insertion and eye superficially striate, rather shiny. Vertex and temple very superficially coriaceus and strongly shiny. Seen from above pronotal dorsum with pair of small lateral tubercles; lateral carinae generally weakly developed. Lateral face of pronotum nearly smooth and shiny. With propodeum in profile posterior margin with several suberect hairs (rarely inconspicuous); propodeal spine more or less distinctly upward-directed with its apex distinctly higher than propodeal dorsum.

L. bedoti: CI 94-100 (96), SL 0.64-0.70 mm (0.67 mm), SI 87-94 (91). Eye distinctly narrowed anteriad, EL 0.13-0.16 mm (0.15 mm), number of ommatidia along long axis 6-7 (rarely 8), EL/EW 1.60-1.83 (1.72). Area between antennal insertion and eye finely but distinctly striate, and mat. Vertex and upper gena weakly sculptured and weakly shiny. Seen from above pronotal dorsum margined laterally with irregular carinae that are darkened. Lateral face of pronotum longitudinally, finely striate, or nearly smooth, and shiny. With propodeum in profile posterior margin generally without suberect hairs; propodeal spine more or less distinctly upward-directed with its apex distinctly higher than propodeal dorsum.

L. striatulus: CI 94-103 (98), SL 0.58-0.62 mm (0.61 mm), SI 84-91 (88). Eye distinctly narrowed anteriad often with pointed apex, EL 10.14-0.16 mm (0.15 mm), number of ommatidia along long axis 6-7, EL/EW 1.63-2.11 (1.87). Area between antennal insertion and eye distinctly striate, and mat. Vertex and upper gena more strongly sculptured and very weakly shiny. Seen from above pronotal dorsum margined laterally with irregular carinae that are darkened. Lateral face of pronotum longitudinally, finely but distinctly striate, and nearly mat. With propodeum in profile posterior

margin generally without suberect hairs (sometimes with a few very fine hairs present); propodeal spine nearly backward-directed with its apex only slightly higher than propodeal dorsum.

Material examined. Holotype: worker, Hon Ba National Park (900 m alt.), Khánh Hóa, S. Vietnam (12˚06'N, 108˚58'E), 20.ii.2014, S. Hosoishi leg., ex leaf litter, SH14-Vie-24, deposited in IEBR (Entomological Collection of the Institute of Ecology and Biological Resources, Hanoi, Vietnam). Paratypes: 14 workers and 2 winged queens, same data as holotype (BMNH, CASC, IEBR, KUM, SKYC). Nontype material: 5 workers, same locality and collector, SH14-Vie-20.

Biological notes. This species inhabits well-developed forests (Figs. 4, 5), and nests in leaf litter under dead wood on the ground.

Figures 4-5. Scenery of collecting sites (900 m alt.) in Hon Ba National Park, Vietnam.

Key to species occurring in South China, Indo-china and Thailand (workers)

1. In dorsal view pronotum anteriorly with pair of lateral spines/teeth (quadrispinosus group). ……2
- In dorsal view pronotum without pair of lateral spines/teeth, but with lateral irregular carinae only (bedoti group). ……3
2. Pronotal spine short, mere denticle. Antennal scape short, with head in full-face view hardly extending beyond posterior margin of head. Taiwan…**L. taivanae Forel**
- Pronotal spine distinctly protruding and apically bluntly pointed. Antennal scape relatively long, with head in full-face view distinctly surpassing posterior margin of head. Thailand, Myanmar, Vietnam, Sri Lanka...……**L. birmanus Emery**
3. Mesosoma extensively smooth and shiny; only upper portion of mesopleuron reticulate. Malay Peninsula, Thailand. …… **L. lucidus Menozzi**
- Mesosoma extensively sculptured; only pronotum often smooth ……..4
4. Antennal scape long, with head in full-face view distinctly surpassing posterior margin of head; SI 100-103. Eye weakly narrowed anteriad with rounded apex; number of ommatidia along long axis of eye 8-9. S. Vietnam…… ……...**L. indosinensis Yamane et Hosoishi**
- Antennal scape shorter, hardly reaching posterior margin of head; SI smaller than 95 (generally ca. 90). Eye strongly narrowed anteriad, often with pointed apex; number of ommatidia along long axis of eye 6-7 (rarely 8)……..……5
5. Lateral face of pronotum and head behind and below eye shiny, at most faintly sculptured. Propodeal spine more or less distinctly upward-directed with its apex much higher than propodeal dorsum. Palawan, Borneo, Sumatra, Malay Peninsula, Thailand, Myanmar, India, Sri Lanka. ……**L. bedoti Emery**
- Lateral face of pronotum and head behind and below eye at least superficially sculptured. Propodeal spine nearly backward-directed with its apex only

slightly higher than propodeal dorsum. Thailand. ..... **L. striatulus Rigato**

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References

Bharti, H. and Kumar, R. 2012. Lophomyrmex terraceensis, a new ant species (Hymenoptera: Formicidae) in the bedoti group with a revised key. Journal of Asia-Pacific Entomology 15: 265-267.

Bolton, B. 1987. A review of the Solenopsis genus-group and revision of Afrotropical Monomorium Mayr. Bulletin of the British Museum (Natural History) (Entomology) 54: 263-452.

Eguchi, K., Bui, T.V. and Yamane, Sk. 2011. Generic synopsis of the Formicidae of Vietnam (Insecta: Hymenoptera), Part I – Myrmicinae and Pseudomyrmicinae. Zootaxa 2878: 1-61.

Eguchi, K. and Yamane, Sk. 2003. Species diversity of ants (Hymenoptera, Formicidae) in a lowland rainforest, northwestern Borneo. New Entomologist 52: 49-59.

Jaitrong, W. and Nabhitabhata, J. 2005. A list of known ant species of Thailand (Formicidae: Hymenoptera). Thailand Natural History Museum Journal 1: 9-54.

Rigato, F. 1994. Revision of the myrmicine ant genus Lophomyrmex, with a review of its taxonomic position (Hymenoptera: Formicidae). Systematic Entomology 19: 47-60.

Sheela, S. and Ghosh, S.N. 2008. A new species of Lophomymrmex Emery (Hymenoptera: Formicidae) from India, with a key to Indian species. Biosystematica 2: 17-20.

Yamane, Sk., Itino, T. and Abd. Rahman Nona. 1996. Ground ant fauna in a Bornean dipterocarp forest. Raffles Bulletin of Zoology 44: 253-262.

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Extended distribution and variation in morphological features of Disparoneura (Chloroneura) quadrimaculata (Rambur, 1842) (Odonata: Zygoptera; Protoneuridae) in the Mt. Abu ranges of Southern Rajasthan, India

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Abstract

Disparoneura (=Chloroneura) quadrimaculata (Rambur, 1842), earlier documented to be endemically present in the mountain ranges of the Western Ghats in southwestern states and the Satpura mountain ranges in Central India, has recently been recorded for the first time near the Nakki Lake in Mount Abu (Alt. 1220 m m.s.l.) range of the Aravalli Hills in south-western Rajasthan, India.

Keywords: Disparoneura (=Chloroneura) quadrimaculata, Mt. Abu, Nakki Lake.

Introduction

Before the genus Chloroneura Laidlaw, 1917 was subsumed into Disparoneura (cf. Davis and Tobin, 1985), it comprised two valid species, i.e., C. apicalis Fraser and C. quadrimaculata (Rambur), both of which originated from India (cf. Fraser, 1933). Biogeographically, D. apicalis is exclusively confined to the lower reaches of the Cauvery River, Coorg (S. India), whereas D. quadrimaculata with a more extensive range occurs in Western Ghats, Coorg, Deccan and Central Provinces (Madhya Pradesh). During a planned expedition with Dr. Peter Miller, a highly celebrated odonatologist in reproductive biology from Oxford University, UK, in the sylvatic mountainous environments of the Nakki Lake in Mt. Abu (Alt. 1220 m m.s.l.), located in southern Rajasthan State, we came across a large number of elegant damselflies which preferred to engage in dance in tandem and basking in Sun on the rocks and vegetation near the Nakki Lake. Due to their striking beauty and rather unusual character of nuptial flight we collected about a dozen specimens, both male and female. On close examination of these specimens it became clear that the species exhibited quite some different traits which need to be brought on record for reason of understanding its geographical expansion in an area hitherto a terra incognito for the species and the taxonomically important changes in morphological features almost approximating to be a different species. We describe in this brief note, the expansion and variation in morphological characters of D. quadrimaculata so as to induce a conservatory scheme for this beautiful creature in the vicinity of a major wetland, the Nakki Lake.

Material and Methodology

A total of 13 D. quadrimaculata specimens were sampled around the Nakki Lake, Mt. Abu (alt. 1220m m.s.l., lat. 24° 6' N, long. 72° 44' E) in the tail of Aravalli mountain ranges in Sirohi District, Rajasthan, India, on September 1st, 1990. These specimens were finally confirmed as D. quadrimaculata by Professor Peter Miller by comparing the specimens with an extensive series of D. quadrimaculata in the British Museum (Natural History), with the help of Dr. Steve Brooks, Curator Odonata Section.

Distinctively variant characteristics were observed in (i) male genitalia, (ii) relatively higher number of postnodals in both the fore-

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and hind wings, (iii) totally dark antennae save for the basal two segments, and (iv) abdominal segments 7-9 black.

Description of male Disparoneura quadrimaculata

Male – Labium pale ; labrum reddish brown: clypeus, frons, occiput, and vertex all dull-red; genae and under surface of head yellowish; labrum with a median black pit almost touching the clypeus; eyes in life bright blood red; antennae 5-segmented, torus and first basal segment reddish, second segment only two-third reddish, darkening apically; rest completely black, antennae angulated at the tip of first basal segment; between the eyes two almost horizontal equatorial black stripes run parallel, the front thinner line arising from the inner edge of the eyes move inwardly and encircles the base of the antennae pronouncedly only on the front half circle, then centering gradually to be thickened mealy; another more thicker line runs rather irregularly between the broadly darkened-base of the eyes, touching the posterior ocelli and extending like an arm from the outer side to faintly meet the bases of the two antennae; the broader strips in fact dive backwards to encircle a large dull red area in the region of vertex, two dark circular spots between the two dark strips near the base of each eye (Fig. 1).

Prothorax dull brick-red mapped with black along sutures and sulci, rear lobes convex; thorax brightly red on the dorsum, turning yellowish on the undersides, and marked with black varyingly (considering the paratypes), stripes on the sutures are always broader along the upper edge; wings hyaline basally and apically, barred with a broad blackish-brown fascia which in the forewings extends from the first postnodal to the ninth postnodal, while in the hindwings begins from the fourth postnodal to the thirteenth postnodal; forewings with 16-18 postnodals and hindwings with 14-15 postnodals; Cu_{ii} 10 to 11 cells long in forewing, 10 to 12 in the hind; pterostigma diamond-shaped with distal nervure more convex, yellowish-brown, strongly bordered, braced and covering only one cell. Legs colored and armored as follows: foreleg femur black on the underside, narrowly and proximally, but gradually broadening distally to cover almost whole of the surface, tibia black only along the base of spiky armature, rest yellowish brown, tarsi totally black, basal tarsus with three short, stout, sharp pointed black spikes curved forwardly and downwardly, middle tarsus with 3-4 similar spikes, apical tarsus with 4-5 spikes; middle leg femur with speckling on the underside extending from base to about halfway only then diffusing into black broadening towards the apex, tibia and tarsi colored as of foreleg, basal tarsus with 3 spikes, middle with 5 and terminal with 4-5 spikes; hind leg femur with the speckling on the underside extending two-third the end, tibia and tarsus similar to the other two legs, basal tarsus with three spikes, middle tarsus with 5-6 and terminal with 4-5 spikes; in all tarsi the last spike is the largest.

Abdomen brick-red, marked with white, brown and black as follows: broad black spot on the dorsum arising from the base but narrowing towards the distal part; segment 2 with a large ring of black on the dorsum touching the base but falling just short of the apex; a narrow longitudinal stripe of black falling similarly short of the apical black rim; segments 3-6 each with a pair of small dorsolateral whitish spots at base, narrowly bordered with black, a narrow ventro-lateral stripe on each side ending short of base and apex, apical one-fifth or so blackish-brown in annules, apical annule of segment 6 jet black; segments 7-9 black, with only base of segment 7 with a highly indistinct and obscured whitish spot; segment 10 reddish dorsally, blackish-brown side wise and below. Anal appendages with superiors reddish-brown dorsally, characteristically notched and inwardly and downwardly bend to form a pointed process in black; inferiors black, obtuse as seen latterly, curled a little inwardly to form acute apices, extending beyond the apices of the superiors as seen from both dorsal and lateral sides (Fig. 2).

Comparing with other species

Disparoneura quadrimaculata (Ramb.) and D. apicalis (Fraser) are the only other two species described under the original genus Chloroneura (cf. FRASER, 1933). However, the collected specimens were most closely related to D. quadrimaculata (Ramb.). Interestingly, on an average our specimens of D. quadrimaculata

Figure 1. Disparoneura quadrimaculata: (a) Head - structure of head showing black stripes on the dorsum; (b) Forewing - pterostigma, and (c) Hindwing - pterostigma.

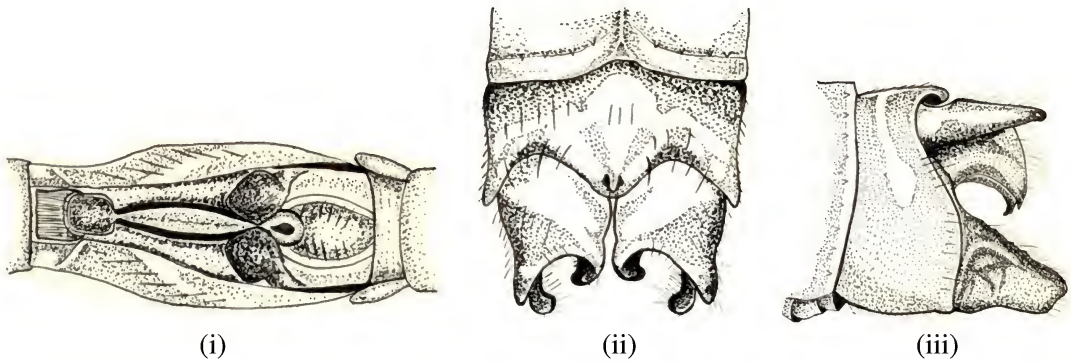

Figure 2. Disparoneura quadrimaculata: (i) Secondary copulatory apparatus seen from the ventral side, (ii) Dorsal view of appendages, and (iii) Lateral view of appendages. Measurements (mm) - total length including cerci 49, abdomen 31, hindwing 21.5.

were slightly smaller in size of body and the wings than those of Fraser's, in forewing and hind wing. In the collected material hind wing is more acutely diamond shaped with the costal nervure markedly longer than its counterpart, the forewing, where the distal nervure is distinctly curved and the costal nervure is only a bit greater in length than its counterpart, but visibly shorter than in the hind wing.

Bioecology

Disparoneura quadrimaculata specimens were always collected near the fast – flowing streams while resting on rocks or vegetation or, as seen some rare cases, on the protruding objects (mostly plant twigs) from the Nakki lake. All specimens were collected on a cloudy day with intermittent rain. (Day temperature 24° C ± 2, and humidity 70% ±10). Males were seen aplenty, as compared to their counterparts. While roosting on a rock they perched close to the surface with the wings held together backwards and partly covering a portion of the abdomen. But when perching on a vertical twig of a plant the wings though held together backwards a projected clearly above the abdomen. Some pairs were seen in tandem as well. The flying males could be more often spotted by the circular ring formed by the darkened middle parts of the wings.

Although some other odonate species, like Ischnura aurora (Brauer), Orthetrum taeniolatum (Schneider), O. pruinosum neglectum (Rambur), O. triangulare (Selys), Trithemis festiva (Rambur) and Brachythemis contaminata (Fabricius) were collected from the

nearby areas, within about 2 km from the Nakki lake, but none really seemed to share habitat with D. quadrimaculata.

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References

Davies, D.A.L. and Tobin, R. 1984. The dragonflies of the world: a systematic list of the extant species of Odonata. Vol. 1. Zygoptera, Anisozygoptera. Utrecht: Societas Internationalis Odonatologica Rapid Comm. (Suppl.) 3:127 pp.

Fraser, F.C. 1933. The Fauna of British India, including Burma and Ceylon. Odonata. Vol. I. London: Taylor and Francis. 419pp.

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Telenomus dilatus sp. n. (Hymenoptera: Platygastridae) - an egg parasitoid of swallowtail butterflies from South India

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Abstract

Telenomus dilatus (Hymenoptera: Platygastridae), an egg parasitoid of swallow tail butterflies is described as new to science. The species has been reared on several occasions in the South Indian State of Kerala, from the eggs of three species of swallow tail butterflies-Troides minos, Pachliopta pandiyana and Pachliopta aristolochiae. T. dilatus sp. n. can be easily distinguished by its basal male antennal segments- A1, A4 and A5, which are extremely dilated. Digital images of the new species are provided and its affinities with closely resembling species are discussed.

Keywords: Telenomus, India, swallowtail butterflies, egg parasitoid, new species.

Introduction

Telenomus Haliday of subfamily Telenominae (Hymenoptera : Platygastridae) is a large cosmopolitan genus of egg parasitoids (Johnson, 1984). The hosts are mostly Lepidoptera and Hemiptera, but they are also known to attack Dipteran and Neuropteran eggs (Johnson, 1984; Johnson and Bin, 1982). With more than 612 described species, this is the largest genus under Platygastridae (Austin et al. 2002). Altogether 22 species of Telenomus are known from India (Rajmohana, 2006, Rajmohana et al. 2013a, 2013b). Though economically significant as biocontrol agents, the systematics of this genus is largely ignored (Johnson, 1984).

The present study describes a new species of Telenomus reared from the eggs of three species of papilionid butterflies, which include, the Indian peninsular endemic, Troides minos (the Southern Birdwing), the south Indian endemic Pachiolopta pandiyana (the Malabar Rose) and also Pachiolopta aristolochiae (the Common Rose), which enjoys a wide distribution in South and South east Asia. The new species described here has been reared on several occasions in the South Indian state of Kerala**.** T. dilatus sp. n. can be easily distinguished by its basal male antennal segments, A1, A4 and A5, which are extremely dilated. Earlier Krishnamoorthy and Singh (1986, 1988) and Veenakumari and Prasanth 1984, Jalali and Singh, 1990 had reported Telenomus species from the eggs of swallowtail butterflies in India.

Materials and methods

This work is a part of the ongoing studies on the systematics of Telenominae in South India. Morphological terminology is after Johnson (1984), Miko et al. 2007 and male genitalia studies follow Polaszek and Kimani (1990). The holotype comparisons of T. stigis Nixon are from the excerpts of the studies (Unpublished) on Nixon's type specimens of Indian Telenominae made by the first author, in 2007, during a study visit to BMNH, London.

Description and light microscopy imaging were done with the help of Leica M205A stereomicroscope and Leica DFC-500 digital camera and images processed using LAS montage. The SEM images were procured with

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Jeol JCM-5000 NeoScope Benchtop SEM, using specimens coated with gold.

All the material studied are deposited in National Zoological Collection, of Zoological Survey of India, Calicut.

Abbreviations and Terminology**:** A1-A11: Antennal segments 1 to 11; T1-T2: Metasomal tergites 1 to 2; L= Length; W= Width; DCI-Dorsal Cephalic Index (ratio of width to length of head measured dorsally; LOL=Lateral ocellar length; POL= Posterior ocellar length

BMNH-Natural History Museum, London; NZC- National Zoological Collection; ZSIC-Zoological Survey of India, Calicut, Kerala.

Systematics

Telenomus dilatus Rajmohana et Anto, sp. n. (Figs. 1-9)

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Material examined**:** Holotype ♀: India: Kerala: Trichur, on 27.vi.2000 (Reg.No. ZSI/INV/4126, reared from eggs of Troides minos. Paratypes 2 ♂ (4127-4128) and 10 ♀ with data same as holotype (4129-4139); 1 ♂ and 1 ♀ (4140 and 4141) emerged on 4.vii.2000 from Pachliopta pandiyana eggs and 2 ♀ (4142-4143) on 1.xii.2010, from eggs of Pachliopta aristolochiae. All specimens reared by Mary Anto from Trichur.

Description

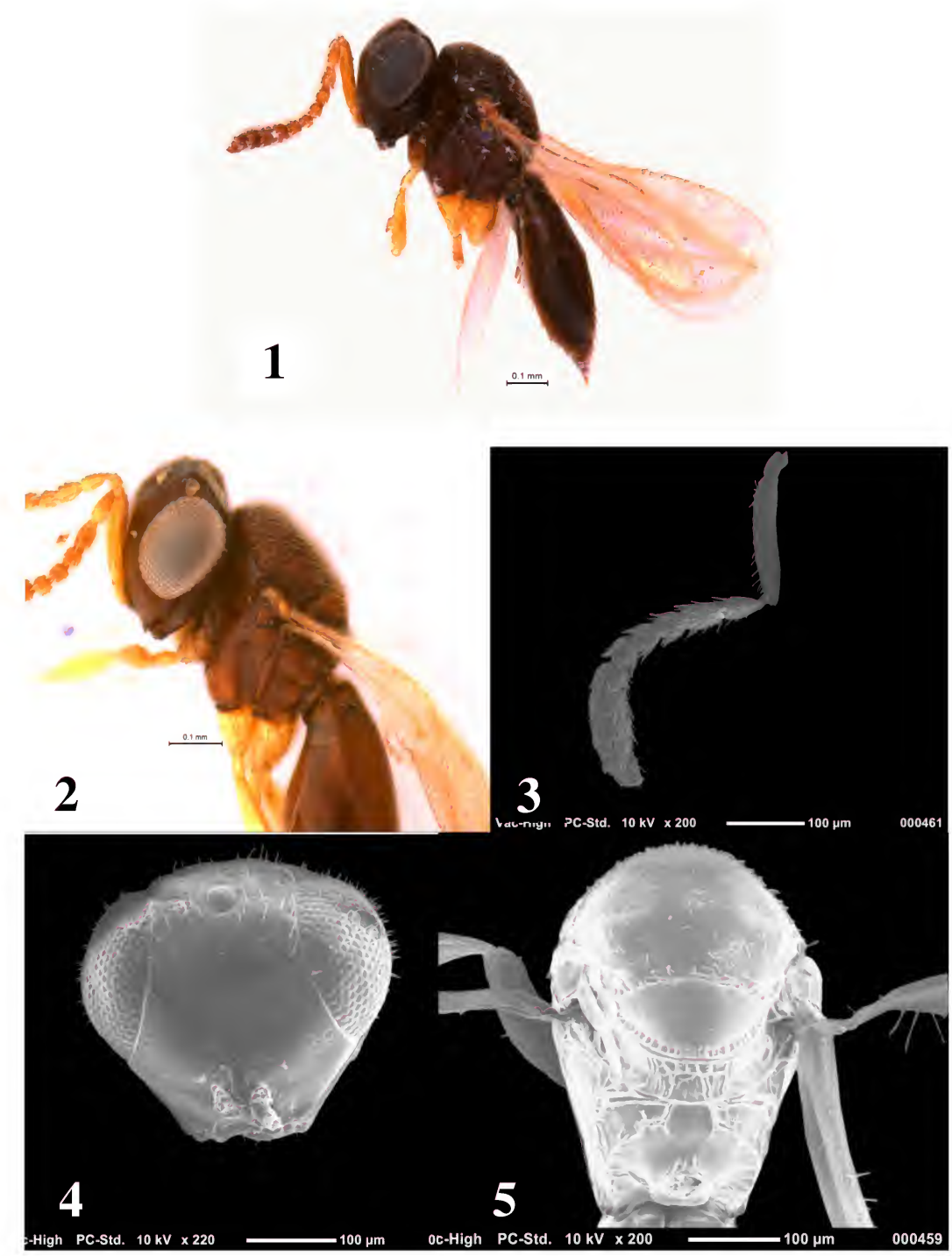
Holotype. Female. Body length =1.01mm. Head and body light to dark brown to black; antennae as well as coxae brownish yellow, fore coxae and claval segments darker; eyes silvery; wings hyaline.

Head: distinctly transverse ; DCI=2.3; vertex and occiput with fine coriaceous reticulate sculpture and with scattered and superimposed setigerous punctures; more coriaceous towards vertex; vertex deeply cut to occiput; hyperoccipital carina seen as a trace; eyes large, densely pilose; occipital carina simple and complete; orbital band wide, not interrupted medially; frons width > eye height(9:8.5); two pairs of ocellar setae distinct; frontal depression weak, frons not bulging between antenna insertions and inner orbit; inner orbits rounded at level of lateral ocelli; LOL: POL= 4.5:10; malar sulcus unusually wide towards orbital corner; temples not bulging laterally; antenna 11 segmented, claval segments 5; A2 2x as long as wide, subequal to A3, A3 length >A4 length (1.2x), A7-A10 transverse.

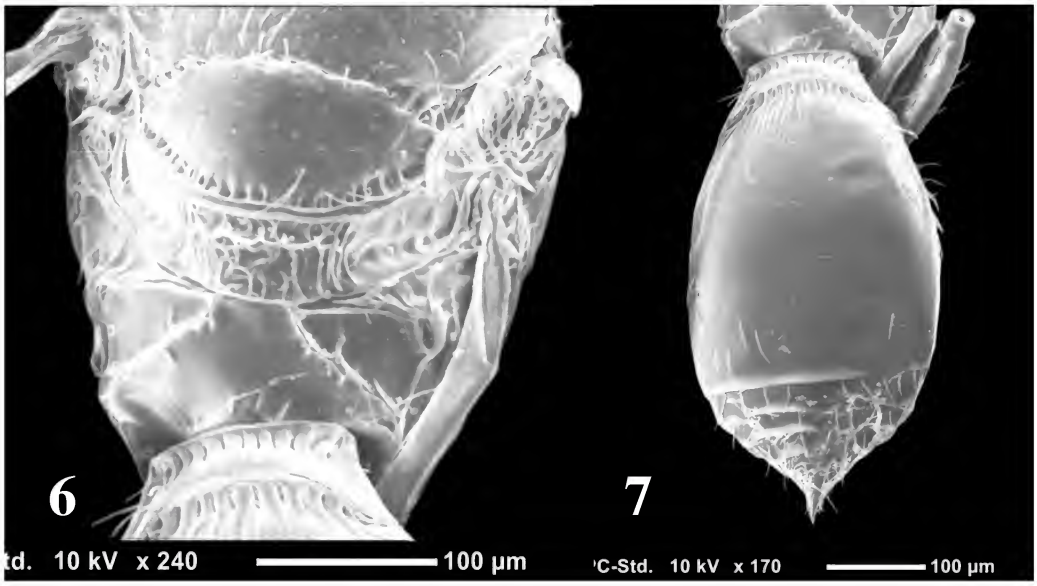
Mesosoma: (L:W =101:95); mesoscutum not as wide as head dorsally (23:26); distinctly convex when viewed laterally, notauli absent; densely setose; sculptured uniformly with rough scaly reticulations; scuto-scutellar sulcus narrow medially, but laterally wide and foveolate; humeral sulcus elongate, not foveolate; mesoscutellum smooth throughout; pubescence not as dense as on mesoscutum; lower margin evenly curved, submarginal foveae smaller than dorsellar punctures; dorsellum longest medially and overlapping propodeum; coarsely reticulate anteriorly, but distally with irregular longitudinal rugosities; acetabular field almost bare; episternal fovea absent; intercoxal space slightly exceeding length of forecoxa; netrion smooth; mesopleural furrow distinct, but mesopleural carina absent; metapleuron bare and smooth medially, except for fine traces of a few crenulae; metapleural carina indicated as a short spur posteriorly; forewing at rest surpassing apex of metasoma; hindwing at its widest point as long as length of marginal fringe; forewing L:W= 26:9; post marginal vein much longer than stigmal vein.

Metasoma: (L:W= 10.2:5.6), slightly less than 2x as long as wide; very slightly longer than combined length of head and mesosoma; T1 with longitudinal striae extending nearly to its three-fourth; 2 pairs of sublateral setae distinct; greatest length of basal costae on T2 along with fine longitudinal wrinkles, 2x median length of T1, extending nearly to dorsal one-fourth.

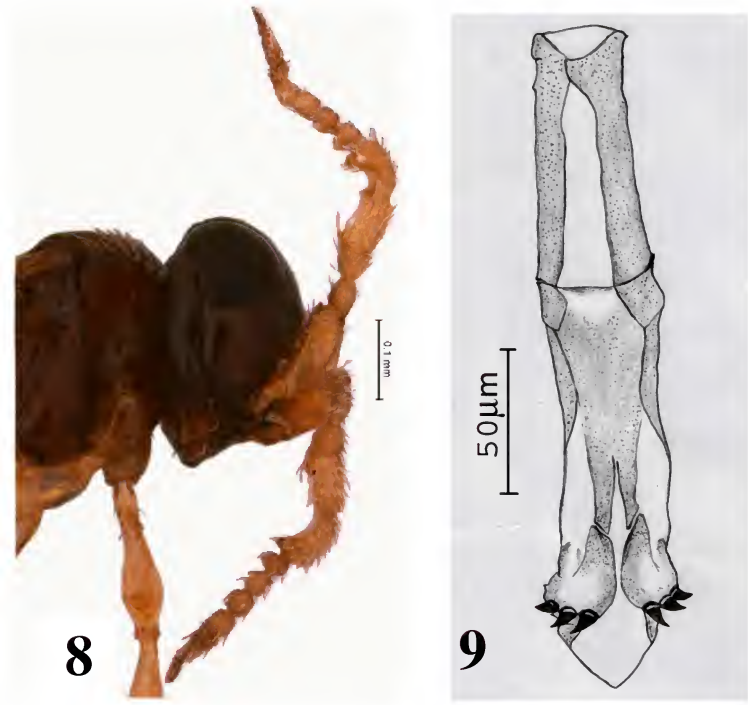
Male: Length 1.03mm. Resembles female, except in aspects mentioned below. Antenna with 12 segments, A1 unusually dilated, wide medially; A4 and A5, subequal, extremely dilated or enlarged, A4 slightly and A5 distinctly curved medially, A6 to A11 more or less globular, A9-A10 transverse; A12 elongate, 1.1x longer than A10 and A11



Figs.1-7: Telenomus dilatus sp. n. (Female) 1.Body Profile; 2. Mesosoma profile; 3. Antenna; 4. Head frontal view; 5. Mesoscutum dorsal view.



Figs.6-7: Telenomus dilatus sp. n. (Female) 6. Mesoscutellum and dorsellum; 7. Metasoma.



Figs.8-7: Telenomus dilatus sp. n. (Male) 8. Antennae; 9. Genitalia.

combined. Male metasoma (L:W= 85:57), widest towards lower T2.

Male genitalia: Resembling much that of T. talus Nixon and T. stigis Nixon as per the medially drawn, tapered and truncate towards tip, one third the length of aedeago volsellar shaft; laminae volsellares sclerotized; digiti large, nearly 0.5x maximum length of aedeagal lobe, with 3 teeth per digitus; central projection absent.

Host: Eggs of swallowtail butterflies- Troides minos, Pachliopta pandiyana and Pachliopta aristolochiae.

Etymology: The species is named 'dilatus' after the dilated appearance of the basal male antennal segments.

Discussion

T. dilatus sp. n. belong to Telenomus californicus species complex, as per Johnson, 1984. Though the females of T. dilatus do not possess any prominent or peculiar distinguishing features, the male antenna with its enlarged A1, A4 and A5 serve as a strong diagnostic character to the species. In addition, the following combination of characters: 11-segmented antenna in females and 12 segmented in males, uniformly reticulate mesoscutum, smooth scutellum, anteriorly reticulate dorsellum, and with irregular longitudinal rugosities distally, T1 with two pairs of sublateral setae, striae on T2 extending nearly to its one-fourth anterodorsally and male genitalia with three teeth on digiti, can distinguish the species.

Nixon 1937 described Telenomus talaus from the eggs of a swallowtail butterfly Papilio agamemnon, collected from Malaysia. The male antenna of this species however do not possess any dilations or enlargements, as met with in T.dilatus sp. n. Nixon 1937 also commented that T. talus was very much similar to T. stigis Nixon, a species reared from the eggs of a moth, Acherontia stynx at Kuala Lumpur (Malaysia) in which A4 and A5 of the male antenna were largely dilated and modified. No other species of Telenomus in Oriental regional is reported to have such a peculiarity. A5 in the males of T. dilatus sp. n. is unique, with a characteristic median curve (A5 in T. stigis is not curved medially), A4 and A5 elongate, almost subequal in length, more than 2x length of A3 in T. dilatus sp. n. (A5 is nearly 1.5x as long as A4 in T. stigis, and A4 is only very slightly longer than A3 in T. stigis). Female antenna is 11 segmented in T. dilatus (in both T. talaus and T. stigis female antenna are 10 segmented only).

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References

Austin, A.D., Johnson N.F. and Dowton, M. 2005. Systematics, evolution, and biology of scelionid and platygastrid wasps. Annual Review of Entomology 50: 553-582.

Jalali, S.K., and Singh S.P. 1990. A new record of Ooencyrtus papilionis (Hymenoptera: Encyrtidae) on the eggs of Papilio demoleus (Linn.) from India. Journal of Biological Control 4(1):59–60.

Johnson, N.F. 1981. The New World species of the Telenomus nigricornis group (Hymenoptera: Scelionidae). Annals of the Entomological Society of America 74: 73-78.

Johnson, N.F. and Bin, F. 1982. Species of Telenomus (Hym., Scelionidae), parasitoids of stalked eggs of Neuroptera (Chrysopidae and Berothidae). Redia 65: 189-206.

Johnson, N.F. 1984. Systematics of Nearctic Telenomus: classification and revisions of the podisi and phymatae species groups (Hymenoptera: Scelionidae). Bulletin of the Ohio Biological Survey 6: 1–113.

Krishnamurthy, A. and Singh, S.P. 1986. Record of the egg parasite, Trichogramma chilonis on Papilio spp. in citrus. Current Science 55(9):461.

Krishnamurthy, A. and Singh, S.P. 1988. Observational studies on the occurrence of the parasitoids of Papilio spp. in citrus. Indian Journal of Plant Protection 16(1):79-81.

Nixon, G.E.J. 1937. New Asiatic Telenominae (Hym., Proctotrupoidea). The Annals and Magazine of Natural History (10) 20: 113-127.

Polaszek, A. and Kimani, S.W. 1990. Telenomus species (Hymenoptera: Scelionidae) attacking eggs of pyralid pests (Lepidoptera) in Africa: a review and guide to identification. Bulletin of Entomological Research 80**:** 57-71.

Mikó, I., Vilhelmsen, L., Johnson, N.F., Masner, L. and Pénzes, Z. 2007. Skeleto-musculature of Scelionidae (Hymenoptera: Platygastroidea) head and mesosoma. Zootaxa 1571: 1–78.

Rajmohana, K. 2006. A checklist of Scelionidae (Hymenoptera: Platygastroidea) from India. Zoos' Print Journal 21(12): 2506-2513.

Rajmohana, K. and Nisha, M.S. 2013a. Telenomus oryzae (Hymenoptera: Platygastridae), a new species of egg parasitoid of the Rice black bug, Scotinophora (Heteroptera: Pentatomidae) from India. Halteres 4 :79-86.

Rajmohana, K., Srikumar, K.K., Bhat, P.S., Raviprasad, T.N. and Jalali, S.K. 2013b. A new species of platygastrid Telenomus cuspis sp. nov. (Hymenoptera), egg parasitioid of tea mosquito bug from India, with notes on its bionomics and mtCo1 data. Oriental Insects 47(4): 226-232, DOI: 10.1080/00305316.2013.871819.

Veenakumari K. and Mohanraj, P. 1994. Life history of Pachiolopta rhodifer (Papilionidae: Troidini) Journal of the Lepidopterists' Society 48(2): 111-120.

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